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| 13. ABSTRACT (Maximum 200 words) <p>Using the hippocampal-slice preparation, we attempted to demonstrate for the first time the operant conditioning of pyramidal cell bursting activity using local micropressure applications of transmitters and drugs as reinforcement; the same injections administered independently of bursting provided a control for direct pharmacological stimulation or facilitation of neuronal firing. The results suggested that the spontaneous activity of individual CA1 pyramidal cells may be reinforced with burst-contingent injections of dopamine D1 and D2 and cannabinoid receptor agonists, whereas CA3 bursting may be reinforced with mu-opioid agonists. Many of these indications of cellular operant conditioning were confirmed at the behavioral level in parallel studies of hippocampal and intravenous self-administration. The results are consistent with the hypothesis that, in the brain slice experiments, drug injections facilitated hippocampal bursting by a cellular mechanism analogous to behavioral reinforcement.</p> | | | |
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OBJECTIVES

The long-term objective of this research is to elucidate the cellular reinforcement mechanisms underlying goal-directed or operant behavior. The specific aim here was to study the properties of a cellular analog of operant conditioning developed originally in a previous grant period. Our idea was to demonstrate, in a reduced (brain-slice) preparation, enhancement of neuronal function by training procedures analogous to operant conditioning. The most important, and indeed defining, property of operant conditioning is the requirement for response-reinforcement contingency; hence, it was obligatory to show that, in our cellular model, increases in neuronal activity were dependent on activity-contingent applications of the reinforcing stimulus. Such cellular changes may be interesting in themselves, but it also was essential to demonstrate their relationship to changes in behavior. As a first step toward providing evidence of such interrelationship, we attempted to show that cellular and behavioral operant conditioning processes exhibit common properties. Accordingly, parallel operant conditioning experiments were conducted at the cellular and behavioral levels in an attempt to reveal the similarities or differences they displayed in the nature of reinforcing agonists and antagonists, reinforcement-related receptors, and brain locations of target cells.

STATUS OF THE RESEARCH EFFORT

The work accomplished in the review period is organized in two main parts--cellular operant conditioning studies and behavioral operant conditioning studies. Most of the research reported was initiated and completed in the present project period. Also included, however, are some projects initiated in a prior period, but completed and published in the present period. A solicited review of reinforcement-related pharmacological receptors, supported in part by this grant, also was written and published in the project period.

I. Cellular Operant Conditioning

- A. Failure of Glutamate to Reinforce Hippocampal CA1 Bursting (Xue, B.G. and Stein, L., Soc. for Neurosci. Abstracts, 16:261, 1990; Xue, B.G., Belluzzi, J.D., and Stein, L., manuscript in preparation).

Work in the previous grant period indicated that hippocampal CA1 bursting may be reinforced by dopaminergic agents such as dopamine itself, cocaine, and certain dopamine receptor agonists. A major concern is that these agents may facilitate bursting merely by direct or indirect pharmacological stimulation of neuronal activity rather than by a cellular reinforcement process. We have always required as critical evidence of cellular reinforcement that noncontingent or random presentations of the positive agents will be relatively ineffective; and indeed random applications of dopamine, cocaine, and dynorphin A are ineffective and even tend to

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suppress the bursting of hippocampal pyramidal cells.

Nevertheless, it may be argued that solutions of neurotransmitters or mimetic compounds applied immediately after a neuronal burst may prevent complete repolarization of the neuron, thereby reducing the transmembrane potential and increasing the likelihood of action potentials occurring in the future. One approach is to monitor the resting transmembrane potential with an intracellular electrode during conditioning experiments. This is a technically difficult approach and, in any case, intracellular recording by other investigators, such as Gribkoff and Ashe (1984) and Malenka and Nicoll (1986), have already shown that the direct effects of dopamine on membrane potential are generally small and inconsistent. Indeed, in cells with extremely stable membrane potentials, Malenka and Nicoll (1986) report that dopamine usually produces a small *hyperpolarization* (consistent with our own findings that noncontingent dopamine applications reduce cellular bursting). Our approach was to test nonspecific depolarizing agents, such as glutamate, for reinforcing activity in the cellular operant conditioning test--this agent, of course, is not associated with the reinforcing properties of drugs in the intact organism. In initial tests, we tested the effects of physiologically-released glutamate induced by local electrical stimulation of the Schaffer collaterals. Applied noncontingently, such stimulation produced a large increase in the rates of CA1 bursting. However, when the same stimulation was applied contingently (as reinforcement for bursting), the excitatory action was substantially diminished. A

similar result was obtained when the electrical stimulus was applied to the molecular layer. In contrast, electrical stimulation of the stratum oriens (the layer in which the dopamine fibers run) facilitated bursting when applied contingently, but inhibited bursting when applied noncontingently. These latter results, of course, mimic the effects of dopamine itself when it is applied both contingently and noncontingently.

As a second approach, we attempted to reinforce hippocampal bursting with direct applications of glutamate. Unlike dopamine and cocaine, burst-contingent micropressure injections of glutamate (0.1 and 0.2 mM) did not produce selective facilitation of cellular bursting when compared to random presentations; indeed, both contingent and random glutamate applications reduced the likelihood of bursts, while at the same time increasing the frequency of individual spikes. These results are consistent with the idea that dopamine's reinforcing action on hippocampal bursting cannot be attributed to nonspecific stimulation. The burst-suppressant action of glutamate is intriguing, and suggests that glutamate mechanisms might normally function in opposition to the dopamine reinforcement mechanisms.

- B. Reinforcement of Hippocampal Burst Activity by the Cannabinoid Receptor Agonist CP-55, 9540 (Xue, B.G., Belluzzi, J.D., and Stein, L., Brain Research, submitted; Xue, B.G. and Stein, L., Soc. for Neurosci. Abstracts 17, 872 (1991).

Involvement of cannabinoid receptors in behavioral reinforcement has been demonstrated in animals by self-administration of Δ^9 -tetrahydro-cannabinol (THC) and in humans by the addictive properties of marijuana and related agents. Furthermore, cannabinoid receptors and reinforcement-relevant dopamine D_2 and mu-opioid receptors are known to share the same signal transduction mechanisms and have in common the ability to activate G_i proteins that inhibit adenylate cyclase. Accordingly, it was of particular interest in this project period to determine whether or not cellular operant conditioning could be demonstrated with cannabinoid receptor activation as reinforcement. The high affinity cannabinoid agonist CP-55940 was used as the reinforcer for CA1 hippocampal operant conditioning (cannabinoid receptors are present in high density in rat hippocampus). Highly reliable CA1 operant conditioning was obtained; more than 55% of the neurons tested were successfully reinforced by burst-contingent applications of CP-55940 (at concentrations of 5 and 10 μM , but not at 2.5 or 100 μM). The same microinjections, administered independently of firing, did not increase bursting rate and therefore provided a control for direct pharmacological stimulation of cellular activity. Co-administration of forskolin (which activates cyclic AMP formation) eliminated the reinforcing action of CP-55940, consistent with the idea that cannabinoid reinforcement may involve inhibition of cyclic AMP formation. The results indicate that cannabinoid receptor activation can reinforce hippocampal CA1 bursting and suggest that cannabinoid receptors,

like dopamine and opioid receptors, may play important roles both in behavioral and cellular operant conditioning.

C. Dopamine D2 and D3 Receptors and Cellular Reinforcement
(Xue, B.G., Belluzzi, J.D., and Stein, L., manuscript in preparation)

Five dopamine receptors are presently recognized, which may be divided on the basis of homology and pharmacological similarity into two main dopamine receptor subgroups, D₁-like (D₁ and D₅) and D₂-like (D₂, D₃, D₄). In early experiments, we showed that the D₂-preferring agonist N-0437 was an effective reinforcer of hippocampal CA1 bursting activity, whereas the D₁ agonist SKF38393 was ineffective. To establish the specificity of N-0437's action at D₂ receptors in this project period, we compared the activity of its optical isomers, N-0923 and N-0924, which differ by 100-fold in D₂ potency. In the dose range 1-6mM, only the D₂-active isomer N-0923 was effective as a reinforcer of CA1 bursting; even at the highest concentration of 6 mM, N-0924 was inactive. The reinforcing action of N-0923 was blocked by co-administration of the selective D₂ antagonist sulpiride (10mM), but not by the D₁-antagonist SCH23,390 (1mM).

We also have conducted CA1 operant conditioning experiments with quinpirole, a D₂-preferring agonist, as reinforcement. Whereas the D₂ receptor is found in the majority of tissues innervated with dopamine, D₃ receptors are present in high densities only in motivationally-relevant limbic forebrain areas.

To our surprise, quinpirole was effective as a reinforcer at 0.025 μ M--approximately 20 times more potent than dopamine itself. Quinpirole has 5 times greater affinity than dopamine for the D_2 receptor, and it also is more resistant to degradation; hence, the 20-fold potency differential is consistent with the possibility that the D_2 receptor subtype plays a major role in the mediation of reinforcement.

D. Dopamine D_1 Receptors and Cellular Reinforcement (Xue, B.G. and Stein, L., Soc. for Neurosci. Abstracts, 1992, in press)

As noted above, we failed in our attempts to reinforce CA1 bursting with the D_1 -agonist SKF38393. However, although regarded as prototypical, SKF38393 is a partial D_1 agonist with only 45% efficacy. A better test of D_1 involvement in cellular reinforcement would be provided by the full agonist SKF82958, an analog of SKF38393. Like SKF38393, SKF82958 produces the rat grooming behavior characteristic of D_1 agonists.

Excellent reinforcement of CA1 bursting was obtained with burst-contingent doses of SKF82958 of 5 and 10 μ M, but not with a higher dose of 20 μ M. When administered independently of bursting, the effective doses of SKF82958 did not increase and often suppressed bursting. Co-administration of the selective D_1 antagonist SCH 23390(+) (10 μ M) eliminated or largely reduced the reinforcing action of SKF82958 (10 μ M). The results indicated that activity-contingent activation of D_1 receptors can reinforce

hippocampal CA1 burst activity, and are consistent with our behavioral data demonstrating that intravenous SKF82958 is avidly self-administered by the rat (see section IID).

II. Behavioral Operant Conditioning

- A. Hippocampal Self-Administration of Dynorphin A (Stevens, K.E., Shiotsu, G., and Stein, L., Brain Research 545: 8-16, 1991).

Unanticipated reinforcing actions of dynorphin A on the bursting activity of hippocampal CA3 cells were discovered empirically in cellular operant conditioning experiments performed in the previous grant period. Dynorphins are not thought to be associated with the behavioral reinforcement produced by opiates; indeed, the kappa receptor--for which dynorphin A has high affinity and is surmised to be a natural ligand--is generally assumed to mediate dysphoria rather than euphoria. Furthermore, no substance has ever been reported to be self-administered in hippocampus (and perhaps none has ever been tried). Thus, although logically derived from the cellular data, the inference that dynorphin A might exhibit behaviorally-reinforcing properties when injected in the CA3 field of hippocampus initially seemed quite improbable. This unlikely prediction nevertheless was verified by the results of hippocampal self-administration work performed in collaboration with Karen Stevens, a recently graduated Ph.D. student trained in our laboratory. Naive rats with cannulas in the hippocampal CA3 region rapidly learned to self-administer dynorphin A, often within

the first few hours of the first test day. Maximum self-administration rates were produced at a dose of 1 pmol/100nl injection; this concentration of dynorphin A is lower than the lowest concentrations of cocaine effective in cortical self-administration experiments by a factor of 50. Addition of naloxone to the dynorphin solutions abolished self-administration behavior, suggesting that dynorphin A exerts its reinforcing effects by actions at hippocampal opioid receptors. In other experiments, more selective opioid antagonists than naloxone were co-administered with dynorphin A to determine the opioid receptor subtype associated with reinforcement. Self-administration was blocked by the selective mu-antagonist B-funaltrexamine, but not by selective kappa or delta antagonists even when administered in high doses. We conclude that mu receptors in the CA3 region of hippocampus are important target sites for opioid reinforcement, as originally predicted by the cellular operant conditioning studies on hippocampal neurons.

- B. Role of Gi/Go Proteins in Reinforcement (Self, D.W. and Pharmacology, Biochemistry and Behavior, submitted for publication; Self, D.W., and Stein, L., Soc. for Neurosci. Abstracts, 1992, in press).

Cellular and behavioral operant conditioning work, in our laboratory and in other laboratories, suggests that dopamine, opioid peptides, and cannabinoids may act as transmitters or modulators in brain reinforcement systems. These transmitters act

at dopamine, opioid or cannabinoid receptors to reinforce cellular firing or whole-animal behavior. The specific dopamine and opioid receptor subtypes involved (D_2 and μ), as well as the cannabinoid receptor, act via pertussis toxin-sensitive G_i/G_o proteins to inhibit adenylate cyclase activity and to enhance potassium conductance. Experiments have been performed in collaboration with David W. Self, a fourth-year graduate student in our laboratory, to investigate the possible role of G_i/G_o proteins and adenylate cyclase inhibition in dopamine- and opioid-mediated reinforcement. These experiments involved both intravenous and intracerebral self-administration of morphine and cocaine. Rats were pretreated with intracerebrally-administered pertussis toxin, in the same discrete brain loci which support drug self-administration, to produce local inactivation of G_i/G_o proteins. If such relatively localized "functional" lesions blocked the reinforcing effects of morphine and cocaine, it would demonstrate that a common mechanism involving G proteins might mediate both dopamine and opioid reinforcement processes.

Despite the technical difficulties associated with intracerebral administration of pertussis toxin, experiments with hippocampal and ventral tegmental area self-administration of morphine have been encouraging. Pertussis toxin was administered in the CA3 region of hippocampus in two concentrations (1.0 or 0.1 μ g). The higher dose produced seizures in all animals, and 50% died 6-7 days after treatment. However, rats injected with the 0.1 μ g dose exhibited no seizures and no differences from saline-

injected controls in body weight or activity. Despite the absence of obvious toxic symptoms, these animals failed to acquire morphine self-administration behavior. Controls injected intrahippocampally with the phosphate-buffered saline vehicle in place of pertussis toxin exhibited rapid acquisition of morphine self-administration. Following a two-week rest period (to allow the effects of the toxin to dissipate), the experimental group was tested in the absence of pertussis toxin. Rapid acquisition of morphine self-administration was observed. These experiments showed that pertussis toxin will prevent acquisition of intracerebral morphine self-administration.

In a second experiment designed to determine the effects of pertussis toxin on the maintenance of morphine self-administration, rats were first trained to reliably self-administer morphine in the ventral tegmental area. After baselines stabilized, a single 500-ng pertussis toxin infusion (1ul over 5 min.) was made in the ventral tegmental area. After allowing 2 days for the toxin to take hold, morphine self-administration tests were resumed with 2-3 days between tests. A subgroup of these rats were concurrently tested for possible toxin-induced changes in response competence. This subgroup, trained previously to respond for food pellets, also had food-reinforcement tests interspersed between morphine self-administration tests. To ensure that any effects of pertussis toxin were due to enzymatic inactivation of inhibitory G proteins, a control group received identical treatment except that heat-inactivated pertussis toxin was substituted for the active toxin.

Active pertussis toxin significantly reduced morphine self-

administration in the ventral tegmental area to 60% of baseline; in the same rats, these injections of toxin did not reduce responding for food pellets, suggesting that the toxin acted selectively to block morphine reinforcement rather than generally to interfere with motor capacity. Inactivated pertussis toxin did not reduce morphine self-administration, demonstrating that enzymatic inactivation of Gi and Go proteins were required for the toxins reward-reducing action. These results support the hypothesis that Gi/Go proteins in the hippocampus and ventral tegmental area mediate the reinforcing effects of opioid drugs.

In a third experiment, we determined whether pertussis toxin would attenuate intravenous morphine and cocaine self-administration. Because intravenous drug injections affect both sides of the brain, pertussis toxin (0.1ug/ul/side) was injected bilaterally in the brain region most commonly associated with stimulant and opioid drug reinforcement--the nucleus accumbens. In control rats, bilateral intra-accumbens injections of heat-inactivated pertussis toxin failed to alter the rate or pattern of high-dose cocaine (0.75mg/kg/injection) or heroin (0.03 mg/kg/injection) self-administration. However, active pertussis toxin produced significant compensatory increases in the self-administration rates of both drugs. (In high-dose drug self-administration, compensatory increases in self-injection rates are regularly observed if the reinforcing effect of the drug is diminished either by dose reduction or administration of antagonists.) The onset of the compensatory increases was delayed

5-6 days after pertussis toxin was injected, consistent with the slow time course of G-protein ribosylation (inactivation) induced by the toxin. Increased self-administration in animals treated with active toxin was initially characterized by highly regular, yet shortened, interinjection intervals, which progressed to bursts of high-rate responding over the next several days. Recovery of baseline performance was not observed even after three weeks. Again, the results support the hypothesis that inhibitory G proteins mediate both stimulant and opioid reinforcement.

C. Dopamine Receptor Subtypes in Behavioral Reinforcement
(Self, D.W. and Stein, L., Soc. for Neurosci. Abstracts,
17, 681, 1992).

The reinforcing properties of the selective D₂ agonists N-0437 and N-0923 were demonstrated for the first time in our cellular operant conditioning experiments. If cellular and behavioral reinforcement mechanisms are interrelated, N-0923 should also serve as an effective reinforcer of behavior. Rats were trained in daily 3-hour sessions to intravenously self-administer cocaine (0.75 mg/kg/injection) by pressing a bar. A second bar delivered no injections and provided a control for nonspecific stimulation. After cocaine self-administration had stabilized, various doses of N-0923 or d-amphetamine were substituted for the cocaine reinforcement. N-0923 was avidly self-administered and, in fact, was substantially more potent than either amphetamine or cocaine. In a second experiment, we attempted to determine the relative

contribution of D_1 and D_2 receptor activation to the reinforcing action of cocaine. Cocaine self-administering rats were pretreated before the test session with either the D_1 agonist SKF 38393 or the D_2 agonist N-0923. It is well established that if a self-administering rat is pretreated with a reinforcement enhancer (such as cocaine itself), the average interval between successive self-administrations is increased and the self-administration rate is decreased; on the other hand, if the pretreatment drug blocks reinforcement, the inter-injection interval is shortened and the self-administration rate is increased. Cocaine self-injections were decreased in a dose-dependent manner by the D_2 agonist N-0923 and increased in a dose-dependent manner by the D_1 agonist SKF 38393. The results support the idea that D_2 , but not D_1 , receptor activation facilitates the reinforcing action of cocaine.

D. The D_1 Agonists SKF 82958 and SKF 77434 are Self-Administered by Rats, (Self, D.W. and Stein, L., Brain Research, 582, 349-352, 1992).

The failure of the prototypical D_1 -agonist SKF 38393 to act as a positive reinforcer constitutes the most important negative evidence against the hypothesis that D_1 receptor activation mediates reward. However, although generally regarded as prototypical, SKF 38393 is only a partial agonist (45% efficacy) and it has only a limited ability to penetrate the blood brain barrier. A better test of D_1 involvement in reinforcement would be provided by SKF 82958, an analog of SKF 38393. SKF 82958 not only

is a full D_1 agonist, but it also has greater ability to penetrate into the brain than its parent compound. In addition, SKF 82958 produces the grooming behavior characteristic of D_1 agonists.

In a first experiment, we determined whether or not drug-naive animals would self-administer SKF 82958. Different groups of rats, trained previously to lever press for food pellets, now received instead an intravenous injection either of SKF 82958 (10 ug/kg) or of the saline vehicle after each lever press response. A total of 15 daily 3-hr test sessions were given. The group receiving SKF 82958 exhibited sustained self-administration throughout the 15 test sessions, while the response rate of the saline controls declined rapidly (presumably, as a result of extinction) from the high levels maintained by the prior food reinforcement. In a second experiment, various doses of SKF 82958 were tested for self-administration using animals that had been trained initially to self-administer either SKF 82958 or cocaine. Each dose of SKF 82958 was offered in two consecutive, 3-hr self-administration tests employing different sequences for each animal; to minimize transition effects, only data from the second test was used for analysis. Finally, these rats were similarly tested with various doses of SKF 77434, an analog that has similar lipophilicity to SKF 82958 and similar partial D_1 agonist efficacy to SKF 38393. Inverted "U-shaped" self-administration dose-response curves were obtained for both SKF 82958 and SKF 77434. SKF 82958 was both more potent and more efficacious than SKF 77434, as indicated by the leftward displacement and higher peak rate of the SKF 82958 self-

administration curve. The inverted "U-shaped" dose-response curves obtained with SKF 82958 and SKF 77434 resemble those seen with many other self-administered compounds, including cocaine and D₂ agonists.

SKF 82958 self-administration was characterized by relatively regular interinfusion intervals, a pattern which also is typical of cocaine. One notable difference is that in the case of cocaine, the beginning of each self-administration session is usually marked by a brief period of rapid response (which, it is speculated, brings blood cocaine levels quickly to preferred levels (Ettenberg et al, 1982)). In contrast, such initial rapid responding was not observed with the D₁ agonists; indeed, in every rat tested, the rate of self-administration actually increased throughout the test session.

These results suggest that the partial efficacy of the prototypical D₁ agonist SKF 38393 is not the decisive factor in its lack of reinforcing efficacy. SKF 77434 has about the same partial D₁ agonist efficacy as SKF 38393, yet we find that it is readily self-administered. It should be noted that both SKF 82958 and SKF 77434 display enhanced lipophilicity when compared to SKF 38393 and therefore penetrate the blood brain barrier more rapidly than the parent compound. Rapid generation into the brain (as produced, for example, by a favorable route of administration) is known to be a determining factor in a drug's reinforcing efficacy (Jaffe 1990). Hence, it is reasonable to assume that the ability of the full D₁ agonist SKF 82958 and the partial D₁ agonist SKF 77434 to support

self-administration behavior is associated, at least in part, with their high lipophilicity. If so, the inability of SKF 38393 to act as a reinforcer would be due, at least in part, to its low lipophilicity.

E. Receptor Subtypes in Opioid and Stimulant Reward (Self, D.W. and Stein, L., Pharmacol. and Toxicology, 70, 87-94, 1992).

An invited review of opioid and dopamine reinforcement receptors was written and published in Pharmacology and Toxicology in the grant period. A unifying biochemical hypothesis of opioid, stimulant, and cannabinoid reinforcement was proposed in the review, based on our cellular and behavioral operant conditioning work and the observation that opioid μ and Δ , dopamine D_2 , and cannabinoid receptors all inhibit adenylate cyclase and stimulate potassium conductance via G_i proteins.

F. Endogenous Opioids and Amphetamine Reinforcement (Trujillo, K.A., Belluzzi, J.D., and Stein, L., Psychopharmacology, 104, 265-274, 1991).

Work initiated in a previous grant period on the role of endogenous opioids in reinforcement function was analyzed and published in this project period. This work showed that very low doses of naloxone, without effect when tested by themselves, can block the reinforcing effects of amphetamine in conditioned place preference. These results provide evidence of interactions between

endogenous opioids and catecholamines in the mediation of reinforcement processes.

PROJECT PUBLICATIONS

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- * Trujillo, K.A., Belluzzi, J.D., and Stein, L. Effects of opiate antagonists and their quaternary analogues on nucleus accumbens self-stimulation. Behavioral Brain Research, 33: 181-188, 1989.
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Trujillo, K.A., Belluzzi, J.D., and Stein, L. Naloxone blockade of amphetamine place preference conditioning. Psychopharmacology, 104: 265-274, 1991.

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Self, D.W. and Stein, L. The D₁ Agonists SKF 82958 and SKF 77434 are self-administered by rats. Brain Research 582: 349-352, 1992.

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Xue, B.G., Belluzzi, J.D., and Stein, L. Reinforcement of hippocampal burst activity by the cannabinoid receptor agonist CP-55,940. Brain Research, submitted.

Xue, B.G., Belluzzi, J.D., and Stein, L. Operant conditioning of hippocampal CA1 neurons with N-0923, a dopamine receptor agonist, as reinforcement. In preparation.

Xue, B.G., Belluzzi, J.D., and Stein, L. Opposite actions of dopamine and glutamate in operant conditioning of hippocampal bursting. In preparation.

* Research completed in prior grant period and published in current period.

PROFESSIONAL PERSONNEL

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* Supported in previous grant period, but data analysis and publications continue into current grant period.

Interactions

(1) Neuroscience presentations (see publications for abstracts).

(2) Consultation with Dr. A. Harry Klopff, Dept. of the Air Force, Wright-Patterson AFB, Ohio. Dr. Klopff and I interact frequently by telephone and occasional visits. We keep each other continually posted on our research results.

Cellular Investigations of Behavioral Reinforcement

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STEIN, L. AND J. D. BELLUZZI. *Cellular investigations of behavioral reinforcement*. NEUROSCI BIOBEHAV REV 13(2/3) 69-80, 1989. —Using the hippocampal-slice preparation, we attempted to demonstrate operant conditioning of pyramidal cell activity using local micropressure applications of transmitters and drugs as reinforcement; the same injections administered independently of bursting provided a control for direct pharmacological stimulation or facilitation of firing. The results suggested that the spontaneous bursting of individual CA1 pyramidal neurons may be reinforced with activity-contingent injections of dopamine and cocaine, whereas, CA3-bursting responses may be reinforced with contingently-applied dynorphin A. We sought to confirm these indications of cellular reinforcement at the behavioral level in studies of hippocampal self-administration (despite the fact that the hippocampus has been ignored as a brain site for chemical self-administration experiments). The results suggested that dynorphin A is a powerful reinforcer of hippocampal self-administration behavior when injected in the CA3 field; experiments still in progress suggest that dopamine can reinforce self-administration behavior when injected in the CA1 field. Successful prediction of new behavioral data from operant-conditioning data at the cellular level helps to validate the cellular data by providing suggestive evidence of interrelationship between cellular and behavioral operant conditioning processes.

Cellular operant conditioning Reinforcement mechanisms Hippocampus Dopamine Dynorphin A

THE problem of characterizing the brain reinforcement mechanism has two main parts. The first is to identify the neural substrate that performs the reinforcing function. We assume this substrate is neurochemically specialized, and shall call these specialized systems the "reinforcing substrate." The second part of the brain reinforcement problem is to identify the neural substrate that is modified by the reinforcement process. Little experimental effort has been devoted to the identification of this substrate, which we shall call the "target substrate."

REINFORCING SUBSTRATES

The idea that reinforcing functions are specialized neurochemically has guided research in this field for more than 25 years (43). The alternative possibility that these functions are not neurochemically specialized has not been disproved, but this view has not proved heuristic. The hypothesis that certain catecholamine and opioid peptide (endorphin) brain systems may serve as reinforcing substrates is supported by evidence from brain self-stimulation and drug self-administration experiments (15, 44, 49, 56). In the self-stimulation experiments (37), animals work to deliver electrical stimulation to their own brains through permanently indwelling electrodes. In the absence of other sources of reward, the reinforcement for self-stimulation behavior must arise from the neuronal activity that is excited by the electrical stimulus. Although such centrally-elicited reinforcement could be an artifact, it more plausibly reflects a natural process. If so, it would be logical to assume that some of the neurons under the electrode tip actually are reinforcing neurons that mediate the effects of natural reinforcers, or at least are neurons that directly or indirectly excite them.

High self-stimulation rates are observed when electrodes are implanted in regions containing catecholamine or opioid peptide cell bodies or pathways. In particular, self-stimulation tightly overlaps the distribution of dopamine cells in the ventral tegmentum and substantia nigra (14). Self-stimulation closely follows the anteriorly projecting dopamine fibers through the hypothalamus, but it correlates somewhat less closely with the dopamine terminal fields in the forebrain (39). Preliminary mapping of enkephalin sites for self-stimulation is consistent with the idea that opioid peptides are involved in self-stimulation (46). Recent mapping of the distribution of dynorphins in the rat brain (18) has revealed rich concentrations of dynorphin cell bodies and fibers in sites known to support very high rates of self-stimulation. It is now possible to speculate, for example, that high rates of self-stimulation in the bed nucleus of the stria terminalis (43), ansa lenticularis (51), ventrolateral periaqueductal central gray and the area just adjacent (28,31), and particularly in the region surrounding the superior cerebellar peduncle immediately lateral to locus coeruleus (32,41) may be associated with their rich dynorphin innervation. The dopamine-opioid peptide reinforcement hypothesis also is supported by pharmacological experiments. Antagonists of dopamine and endorphins, such as haloperidol and naloxone, respectively, should block chemical transmission of reinforcement messages. In support of the model, there are many reports that these drugs selectively block self-stimulation (3, 21, 26, 43).

In self-administration experiments, behavior is reinforced by central or systemic injections of neurotransmitters or drugs. Of thousands of chemical substances available, animals and man avidly self-administer only a few. These self-administered substances may properly be termed pharmacological reinforcers, and

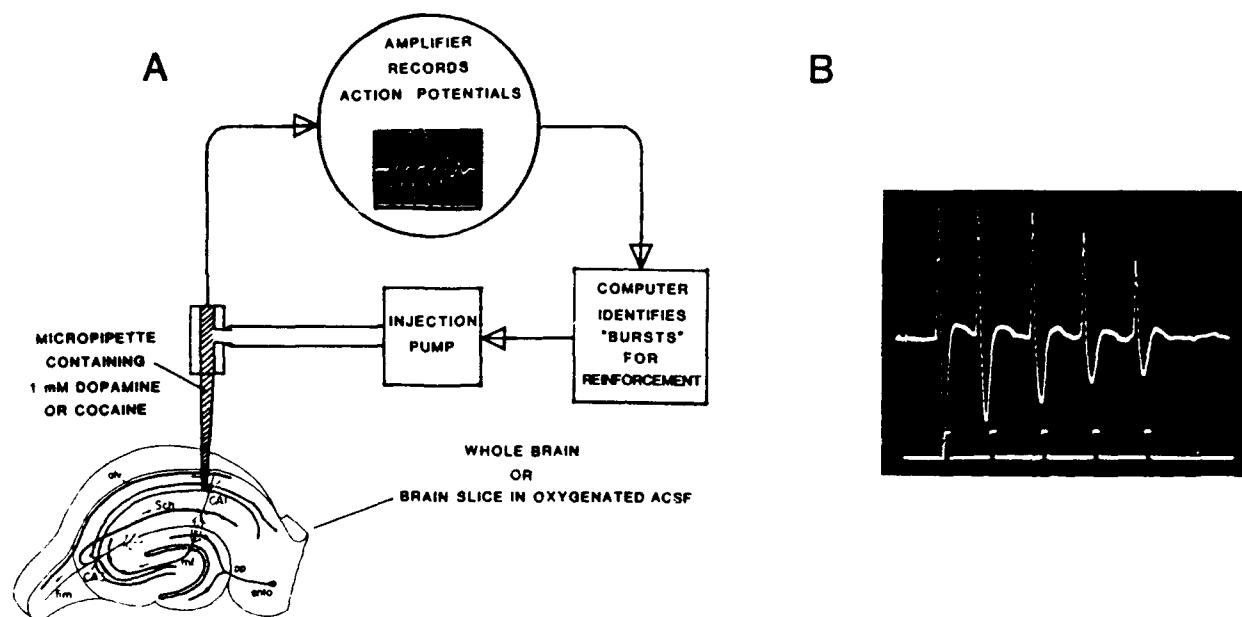


FIG. 1. A) Schematic diagram of cellular operant-conditioning experiment. A single-barrelled glass micropipette for simultaneous recording and pressure injection is filled with dopamine (1 mM in 165 mM saline) or other drugs and aimed at spontaneously active hippocampal cells in the CA1 layer. Amplified action potentials are processed by a spike enhancer and window discriminator (not shown) to increase the signal-to-noise ratio and to isolate signals when multiple-unit activity is encountered. When the computer recognizes a reinforceable burst of activity (based on criteria established individually for each test neuron before operant conditioning), the pressure-injection pump is activated for 5–50 msec to deliver an approximately 10 to 20 μ -diameter droplet of drug in the close vicinity of the cell. Drug-induced increases in bursting are necessary but not sufficient evidence of cellular operant conditioning, since the chemical treatments may directly stimulate or facilitate cellular firing. As a mandatory control for such pharmacological stimulation, the same drug injections are also administered independently of bursting on a noncontingent or "free" basis. Cellular-reinforcing effects may be inferred only if the noncontingent injections are relatively ineffective (48). B) (Upper trace) Burst of firing recorded extracellularly from a CA1 cell, exhibiting typical decrescendo pattern with progressively shorter and broader spikes occurring later in the burst. (Lower trace) 1-msec logic pulses. Spikes that satisfy the preset amplitude criteria of the discriminator are converted to logic pulses for counting by the computer.

it is interesting to ask why these chemicals are selectively associated with behavioral reinforcement. It may be no coincidence that most powerful pharmacological reinforcers have the ability to mimic or release the hypothesized natural reinforcing transmitters. Thus, many dopamine and opioid receptor activators are known to support self-administration behavior. In particular, the naturally occurring opioid peptides Leu- and Met-enkephalin and certain degradation-resistant analogs will support self-administration behavior when injected both intraventricularly (3) and directly into particular brain sites (22,38). These reinforcing effects are blocked by naloxone (22). Similarly, the dopamine receptor agonists apomorphine and piribidol are self-administered systemically (2), and dopamine itself is self-administered directly into the nucleus accumbens (17).

TARGET SUBSTRATES

As noted above, little consideration has been given to the identification of the substrate that is modified by the reinforcement process. What are the neural targets of the reinforcing system? Since it is behavior that is reinforced, it is plausible to assume that behavioral substrates are major targets. A behavioral response obviously reflects the activity of many neurons. Is it the integrated activity of these neurons that is reinforced; that is, is positive reinforcement exerted at the level of neuronal system? Or is it the individual activities of the relevant neurons that is reinforced; that is, is positive reinforcement exerted at the cellular level?

It is commonly believed that positive reinforcement is exerted at the systems level, and probably involves the strengthening or reorganization of the neural substrate of the whole response. The sheer number and virtually infinite variation of reinforceable operant behaviors, however, makes it unlikely that the substrate of the whole response is the brain unit for reinforcement. Furthermore, whether or not particular behavioral variations are treated as one response, or as different responses, depends on the reinforcement contingencies. Thus for example, if lever-press behaviors of 5-gram and 10-gram force are reinforced indiscriminately, both are counted as the same "correct" response; however, if they are selectively reinforced, the behavioral variations are clearly regarded as different responses. The fact that closely similar behavioral variations may be reinforced either indiscriminately or selectively suggests that what is reinforced is not the whole response itself, but specific behavioral features or response elements. In view of the high behavioral resolution that can be achieved by differential reinforcement, these response elements must have a fine grain and, presumably, a correspondingly microscopic neural substrate. Such considerations, in conjunction with the theoretical work of Klopff (29) and the impressive explanatory power of current cellular models of classical conditioning (27), have led us to consider seriously the possibility that individual neuronal activity may be directly modified by reinforcing stimuli (4,50).

Olds (36) was the first to report apparent evidence for the operant conditioning of single neurons. In these experiments,

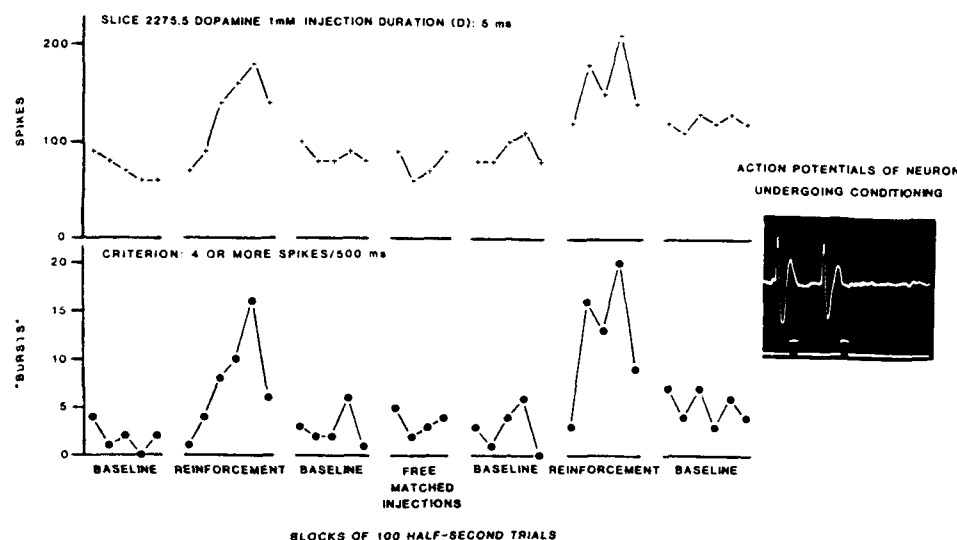


FIG. 2. Operant conditioning of the activity of a CA1 pyramidal cell in a slice of dorsal hippocampus with local injections of dopamine used as reinforcement. The activity of the unit throughout seven phases of a complete experiment is shown. Each point shows the number of bursts (lower graph) and the total number of spikes (upper graph) in successive blocks of 100 half-second samples or trials. Prior to the first baseline phase, a burst criterion of 4 or more spikes per half-second sample was selected. This criterion gave a burst rate for this unit that never exceeded 4 percent in the initial baseline period (BASE). In the reinforcement period (REINF), dopamine HCl (1 mM in 165 mM saline) was applied for 5 msec immediately after each burst. Following a second baseline period, the same dopamine injections were delivered (MATCH) independently of the unit's behavior as a control for possible stimulant effects. The number of injections was matched to that earned during the last four periods of the reinforcement phase. Rates of bursting and overall firing were increased by the contingent dopamine injections during the reinforcement periods, but were not increased when the same injections were administered noncontingently in the matched-injection period. Inset: (upper trace) photograph of oscilloscope display of two action potentials from the unit undergoing conditioning, and (lower trace) 1-msec logic pulses (48).

freely-moving rats with implanted microelectrodes received food or rewarding brain stimulation contingent on appropriate bursts of single-unit activity. Firing rates were increased in a number of cases, suggesting reinforcement of the single-unit response. Unfortunately, it is not clear whether it was the behavior of the individual neuron that was being reinforced or whether some more complex response or movement, of which the neuron's activity was a part, was actually being reinforced. In some of Olds' tests a restriction system was used to limit movement: electronic detectors were discharged by most movements and these precluded reinforcement. Although operant conditioning was still obtained under these conditions, one cannot rule out the possible reinforcement of behaviors involving undetected movements, such as postural adjustments or attentional responses. Like other investigators who have attempted to demonstrate operant conditioning of single-unit activity (19,57), Olds recognized that, if a reinforcing stimulus is delivered to a behaving animal, it is impossible to separate the reinforcement of single units from the reinforcement of more complex responses.

Our solutions to this problem are 1) to use a greatly reduced experimental preparation—the brain slice, and 2) to deliver the reinforcing stimulus—a microinjection of dopamine or cocaine—locally to the neuron being conditioned (4, 5, 47, 48, 50). For our initial experiments, the hippocampal brain slice had many advantages. First, due to a fortuitous anatomy, the hippocampus can be cut into slices which preserve the viability and activity of the neurons in the intact structure (1). Neurophysiological studies show that the electrical activity recorded from slices is comparable to that obtained from an intact preparation (42). The hippocampus is the target of putative dopamine and endorphin reinforcing systems. Dopamine projections to hippocampus are now well

established (8) with the presubiculum-CA1 field as the main target area (55); the hippocampus furthermore is rich in dopamine receptors and dopamine-sensitive adenylate cyclase (16), and the relative density in hippocampus of dopamine D2 receptors in relation to D1 receptors is the second highest in the brain (11). The hippocampus also is well-innervated by enkephalin projections (20), and a rich dynorphin projection—from dentate granule cells to CA3 dendrites—via the mossy fiber system has recently been described (13,18). The hippocampus also is rich in mu, delta and kappa opiate receptors (34).

Consistently with the above described anatomy, we have been able to demonstrate apparent "operant conditioning" of hippocampal CA1 single-unit activity with local administrations of dopamine, cocaine, or the dopamine D2 agonist N-0437 as reinforcement, and similar operant conditioning of CA3 single-unit activity with local administrations of dynorphin A₁₋₁₇ as reinforcement. Application of the reinforcing substances had opposite effects on subsequent firing rates, depending on the activity pattern of the neuron at the time of drug administration. If the neuron had been firing rapidly just before the injections, the firing rate was increased; if the neuron had been firing slowly or was silent at the time of the injection, the firing rate was unaffected or decreased. These observations, therefore, are consistent with the possibility that the activity of individual neurons may be operantly conditioned by direct cellular applications of reinforcing transmitters or drugs.

Although some of these observations have been published in preliminary form (4, 5, 47, 48, 50), they will be reviewed in more detail in the present paper. But even if the operant conditioning of single units can be demonstrated, does such a cellular process actually contribute significantly to behavioral operant condition-

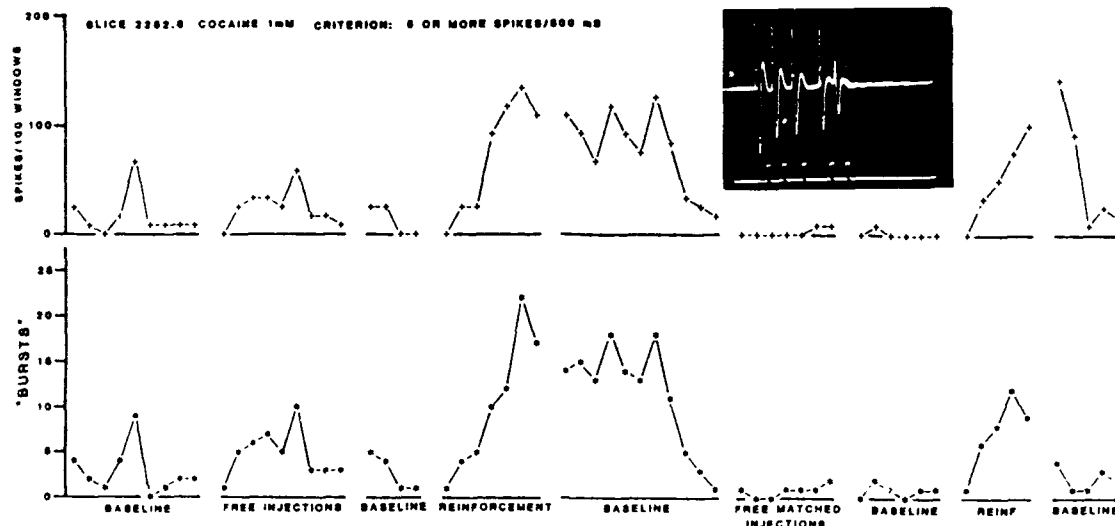


FIG. 3. Operant conditioning of a CA1 pyramidal neuron in a dorsal hippocampal slice using local injections of cocaine as reinforcement. For details, see text and Fig. 2. FREE = noncontingent injections (48).

ing? As a first step toward providing evidence of such interrelationship, it may be possible to show that cellular and behavioral operant-conditioning processes share common properties. Indeed, as an even stronger test, one might try to predict new behavioral operant-conditioning data from observations at the cellular level, and vice versa. In the present research, parallel operant-conditioning experiments have been conducted at the behavioral and cellular levels in an attempt to reveal the similarities or differences they may display in the nature and interrelation of reinforcing transmitters and drugs, reinforcement-relevant receptors, and brain locations of target cells.

CELLULAR OPERANT-CONDITIONING STUDIES

METHODS

In these experiments, operant-conditioning procedures are used in an attempt to increase the firing rates of individual neurons in hippocampal slices. Microinjections of reinforcing transmitters or drugs are applied directly to the cell after bursts of neuronal firing. Increased neuronal activity following contingent drug applications is taken as evidence of operant conditioning or cellular reinforcement if, and only if, noncontingent applications of the same treatments are relatively ineffective. The experimental protocol is diagrammed in Fig. 1. Initially, a somewhat arbitrary decision was made in choosing which aspect of unit activity to reinforce. Since firing rates are likely to be an important vehicle for information transmission, peak rates should have high information value and might be amenable to conditioning. Thus, in early experiments, a half-second period of relatively rapid activity was defined as the neuronal response to be reinforced. These neuronal responses or "bursts" were individually determined for each unit studied. Prior to the start of conditioning, 500 successive half-second samples of neuronal activity were recorded and a frequency distribution of the number of spikes per sample was compiled. A "burst" was defined as that spike number equalled or exceeded in only 2–6 percent of the samples. During operant conditioning, reinforcements were delivered at the end of the half-second time sample containing such bursts of firing. To minimize injection artifacts, neuronal activity during and for 3 sec after each injection was

excluded from analysis and had no programmed consequences.

In later experiments, after it appeared that bursting per se might be amenable to conditioning, the computer program was modified to permit explicit detection of bursts of firing. In the modified program, a burst is defined as a train of firing containing n or more spikes with a maximum interspike interval (ISI) of t msec (a spike-counting program accumulates successive spikes occurring within t msec and recognizes a burst if the total equals n or more). For most units, $n = 3-7$ and $t = 10-15$ msec; in the example shown in Fig. 1, $n = 5$ and $t = 10$ msec. Again, parameters were set individually for each test neuron so that, on baseline, bursts occurred at a rate of approximately 2–6 per min. Because the modified program explicitly detects the occurrence of bursts, reinforcements could be programmed to coincide almost precisely (i.e., within t msec) with the termination of bursts or to follow bursts after specified delays.

The cellular operant-conditioning method involved six stages: 1) Baseline: The number of bursts in the absence of reinforcement (operant level) was determined during a baseline period of approximately 10 minutes. 2) Operant Conditioning: Each burst was now followed by an injection of the reinforcing solution. If conditioning failed to occur after 5 minutes, the duration of the injection (and hence the dose) was increased until evidence of conditioning was obtained, or until direct pharmacological or mechanical effects interfered with recording. 3) Extinction: Reinforcement was terminated, and recording continued until the baseline was recovered. 4) Matched "Free" Injections: Noncontingent injections of the reinforcing solution were made at regular intervals to determine their direct pharmacological effects on rates of firing and probability of bursts. The number of "free" injections per minute was matched to the rate of reinforcing injections in the preceding phase of operant conditioning. Occasionally, a burst would occur within 500 msec of a programmed free injection; on these occasions, in order to minimize adventitious reinforcement, the programmed injection was delayed 500 msec. 5) Washout: A second baseline period without injections was given in order to allow residual effects of the noncontingent drug administrations to be dissipated. 6) Reacquisition: A second period of reinforcement was scheduled, whenever possible, in order to compare rates of original acquisition and reacquisition.



FIG. 4. Chlorpromazine blocks dopamine-reinforced operant conditioning of hippocampal CA1 cellular activity in brain slices (see the Method section and Fig. 2 for procedure). Bars show peak rates of bursting, which are calculated by averaging the two highest 100-trial (or 50-sec) bursting scores recorded for each unit, and then averaging for the treatment group. Neurons reinforced with 1 mM dopamine (DA-REINF) exhibited significantly higher bursting rates than controls reinforced with saline (SAL-REINF). When 1 mM chlorpromazine was added to the dopamine solution (DA + CPZ), the reinforcing action of dopamine was abolished and the bursting rate was suppressed below the saline control. Neurons that had received chlorpromazine alone as reinforcement (CPZ) exhibited the same number of bursts as those that had received saline. SAL-FREE = noncontingent saline injections; DA-FREE = noncontingent dopamine injections. Number of cells in each treatment group indicated in parentheses. *Differs from SAL-REINF, $p < 0.05$.

Evidence of Cellular Operant Conditioning

Results from a positive experiment with dopamine used as the reinforcing agent are shown for a hippocampal unit in Fig. 2. In two separate periods of operant conditioning (REINF), the frequency of bursts and the overall firing rate were rapidly increased after approximately 5 dopamine reinforcements. The same dopamine injections administered noncontingently (MATCH) failed to increase either burst frequency or overall firing rate. Because neuronal activity was not increased by these noncontingent administrations, we can rule out the possibility that direct stimulant effects of dopamine caused the increases in neuronal activity that were observed in the reinforcement periods; indeed, neurophysiological studies almost invariably report decreased activity of CA1 cells after local microiontophoretic or micropressure application of dopamine [for review, see (8)]. Accordingly, we tentatively attribute these drug-induced increases to a cellular process akin to operant conditioning. It also may be seen in Fig. 2 that rates of bursting and overall firing declined sharply after reinforcement had been terminated, suggesting rapid extinction of neuronal operant conditioning. Other units, however, sometimes respond for protracted periods in extinction (e.g., Fig. 3).

Note also in Fig. 2 that the firing rate turned down at the end of both reinforcement periods. This effect typically is observed if high rates of bursting have been generated by the reinforcement procedure, and we tentatively attribute it to a direct inhibitory effect of dopamine when the reinforcement density (and therefore the local dopamine concentration) is excessive. In an effort to protect the unit from excessive dopamine concentrations, we typically terminate the reinforcement period at the point that the

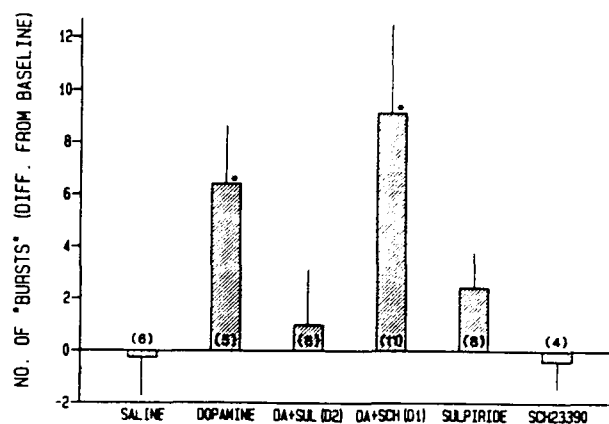


FIG. 5. The selective dopamine D2 receptor antagonist sulpiride, but not the selective D1 antagonist SCH23390, blocks CA1 cellular operant conditioning in hippocampal slices with local injections of dopamine used as reinforcement (see the Method section and Fig. 2 for procedure). Neurons reinforced with 1 mM dopamine (DOPAMINE) exhibited significantly more bursts than controls reinforced with saline (SALINE). When sulpiride (10 mM) was added to the dopamine solution (DA + SUL), the reinforcing action of dopamine was abolished and the rate of bursts was suppressed to the saline control level. On the other hand, when 1 mM SCH23390 (1 mM) was added to the dopamine solution (DA + SCH), the reinforcing action of dopamine was unaffected. Number of cells in treatment group indicated in parentheses. *Differs from SALINE, $p < 0.05$.

acquisition curve turns down. In this regard, mention should be made of the fact that a relatively high concentration (1 mM) of dopamine was required for reinforcement. However, it should be kept in mind that the total drug dose is determined not only by the concentration of the solution in the micropipette, but also by other parameters, such as injection duration and volume. Because drug injections in this experiment had to be delivered to individual cells in close contingency to bursts of activity, it was necessary to use exceedingly short injection durations (5–50 msec) and small volumes (0.5–5 picoliters). After diffusion to action sites, these minute droplets of drug presumably are diluted to concentrations comparable to those produced in more conventional neuropharmacological or brain self-administration studies, where lower initial concentrations of drug are applied in much greater volumes and for much longer durations. In any case, until more is known about the local distribution and metabolism of the reinforcing agents, our strategy has been to determine effective reinforcing concentrations empirically and to compare these concentrations with identical control concentrations applied noncontingently or after a delay. Nonspecific effects that may be associated with the reinforcing injections also can be assessed and ruled out by mixing the same drug concentrations with specific receptor antagonists and showing that such mixtures are ineffective (e.g., Figs. 4 and 5).

Results from a positive experiment with cocaine as reinforcement are shown in Fig. 3. Initially, free injections of cocaine delivered at a rate of approximately 5 per minute had no effect on the frequency of bursts or on the overall firing rate. In the first reinforcement period, after approximately 10 applications of cocaine, the frequency of bursts and the overall firing rate were sharply increased; again, both curves turned down at the end of the period, presumably because of an excessive local cocaine concentration. Unlike the experiment shown in Fig. 2, neuronal firing rates in the baseline period that followed the first phase of

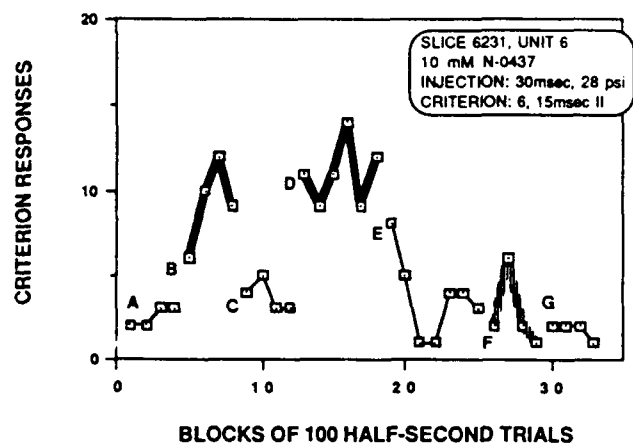


FIG. 6. Operant conditioning of a CA1 pyramidal neuron in a dorsal hippocampal slice with local injections of the selective dopamine D2 agonist N-0437 used as reinforcement. Heavy solid lines at B and D indicate reinforcement periods, during which criterion responses or bursts (6 or more spikes with a 15-msec interspike interval) were followed by drug injections; heavy broken line at F indicates a period of noncontingent injections, matched in number to those earned in corresponding trials in period D. For details, see text and Fig. 2 (9).

reinforcement did not extinguish rapidly; indeed, the peak-firing rates achieved in the reinforcement phase were sustained for several minutes after the onset of extinction. Free cocaine injections ("MATCH") then were delivered at a rate of approximately 12 per minute to match the peak rate obtained in the preceding reinforcement period. These densely-packed free injections had no effect on the number of bursts or on the overall firing rate. In a second reinforcement period, contingent injections of cocaine again increased the frequency of bursts and the overall firing rate, but not to the level observed in the first reinforcement period. In other experiments, saline was substituted for dopamine, or dopamine was administered noncontingently throughout the experiment. In these experiments, neither bursting rates nor overall firing rates were increased [see (50) for a summary of these early experiments].

Evidence of Dopamine Receptor Specificity

Dopamine receptor antagonists were studied in cellular operant-conditioning experiments in an attempt to determine whether dopamine's reinforcing action is specifically exerted at a dopamine receptor or is due to some nonspecific action of dopamine (5). In initial experiments, the mixed dopamine D1/D2 receptor antagonist chlorpromazine completely blocked dopamine's reinforcing action in cellular operant conditioning (Fig. 4). In these experiments, CA1 hippocampal units reinforced with dopamine (DA-REINF) again exhibited significantly higher peak-bursting rates than control neurons reinforced with saline (SAL-REINF). When chlorpromazine was added to the dopamine solution (DA + CPZ), the reinforcing action of dopamine was abolished; indeed, the dopamine-chlorpromazine mixture apparently suppressed the rate of bursting below the saline control and below those neurons that had received chlorpromazine alone (CPZ) as reinforcement.

The availability of new drugs with greater selectivity than chlorpromazine has enabled us to distinguish between effects exerted at dopamine D1 and D2 receptors (Fig. 5). When the selective D2 antagonist, sulpiride, was added to dopamine (DA + SUL), the reinforcing action of dopamine was abolished and the

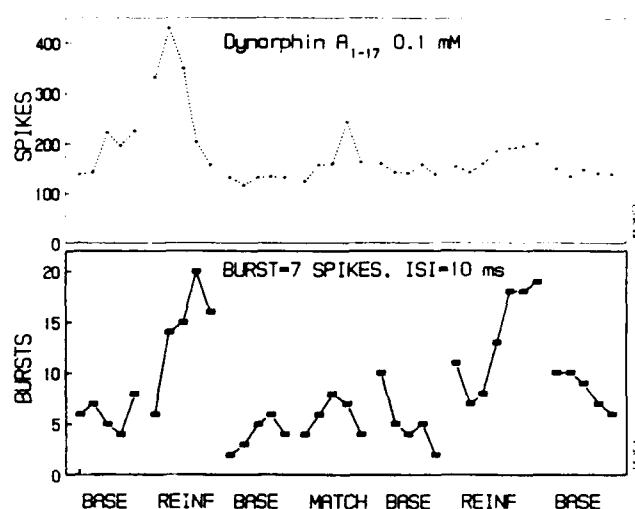


FIG. 7. Operant conditioning of a CA3 pyramidal neuron in a dorsal hippocampal slice with local injections of dynorphin A used as reinforcement. Note selective reinforcing action of dynorphin A on rates of bursting (lower graph), but not on overall firing rates (upper graph). For this unit, bursts were defined as trains of 7 or more spikes with an interspike interval (ISI) of 10 msec. For details, see text and Fig. 2.

rate of bursts was suppressed to the saline control level. On the other hand, when the dopamine D1 receptor antagonist, SCH23390, was mixed with dopamine (DA + SCH), the reinforcing action of dopamine was unaffected or possibly even slightly increased. These results suggest that dopamine's reinforcing effects on CA1 cells are exerted at dopamine D2 receptors. This conclusion is supported by positive experiments with the D2 receptor agonist, N-0437, which may be substituted for dopamine as an effective reinforcer in neuronal operant conditioning (Fig. 6). N-0437 has been found to be a highly reliable reinforcing agent, although it is necessary to use higher concentrations of N-0437 than dopamine (10 vs. 1 mM, respectively). The reinforcing action of 10 mM N-0437 was completely blocked in mixtures containing 1 mM chlorpromazine.

Opiate Reinforcement of CA3 Units

Although hippocampal CA1 activity may be reinforced by

TABLE 1
REINFORCING EFFECTS OF DYNORPHIN A₁₋₁₇ IN CELLULAR OPERANT-CONDITIONING EXPERIMENTS

| Dynorphin A Pipette Concentration (mM) | Peak Bursting Rates of Hippocampal Units in Reinforcement Periods as % of Baseline | |
|---|---|------------------|
| | CA3 | CA1 |
| 0.033 | 72.0 ± 3.2 (4) | |
| 0.1 | 246.73 ± 46.5* (9) | 67.9 ± 13.5* (6) |
| 0.33 | 86.9 ± 6.6 (3) | |
| 0.1 (+1 mM naloxone) | 109.3 ± 4.7 (3) | |

*Significantly different from baseline, $p < 0.05$.
Number of cells tested shown in parentheses.

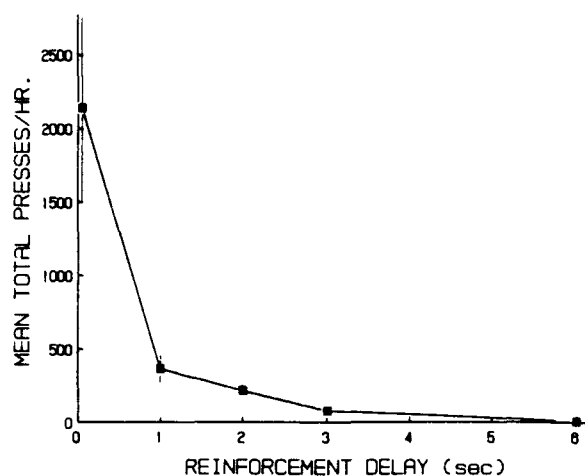


FIG. 8. Acquisition of operant behavior (medial forebrain bundle self-stimulation) as a function of reinforcement delay. Total lever-press responses on Day 1 of training are shown for different groups of rats reinforced after the indicated delay. Note that a delay of only 1 sec produced a rate decrease of approximately 90%. Vertical lines represent \pm S.E.M. [After (10)].

cellular applications of dopamine, cocaine or N-0437, the same dopaminergic drugs have failed, in preliminary attempts at least, to reinforce the activity of CA3 cells. Recent mapping of the distribution of dynorphins in rat brain (18) has revealed high concentrations in sites known to support high rates of self-stimulation, suggesting the possibility that self-stimulation behavior at these sites may be associated with the release of dynorphins. For this reason, and because the principal dynorphin fiber tract in hippocampus terminates in the CA3 field, we attempted to

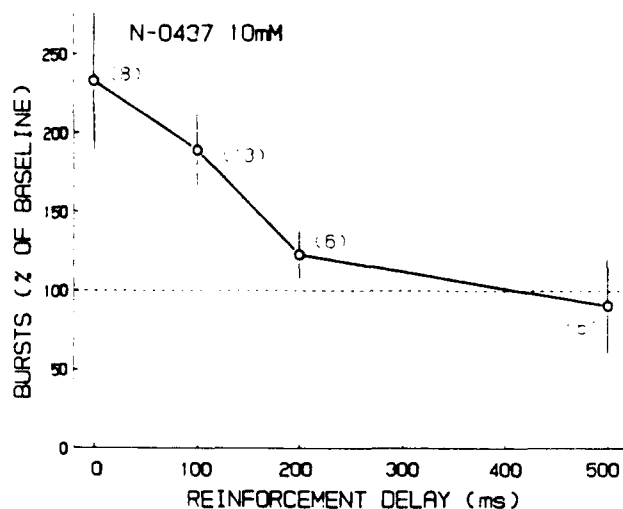


FIG. 9. Delay of reinforcement gradient in neuronal operant conditioning with N-0437 (10 mM) as reinforcement. Compare with behavioral delay of reinforcement curve shown in Fig. 8. Number of neurons tested at each reinforcement delay indicated in parentheses. Vertical lines represent \pm S.E.M. (6).

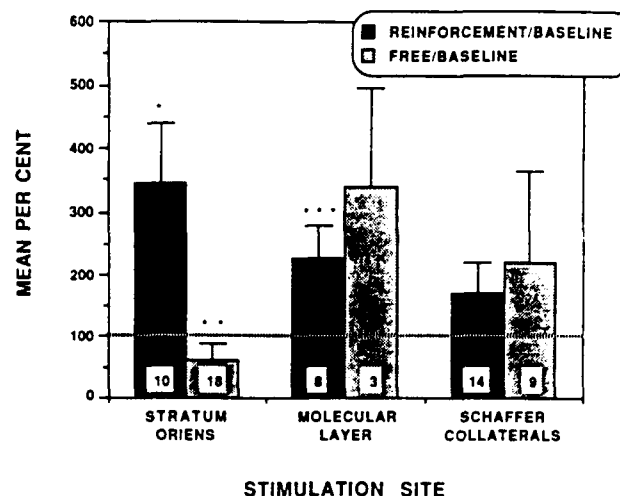


FIG. 10. Summary data of CA1 cellular operant-conditioning experiments in hippocampal slices with locally-applied electrical stimulation used as reinforcement. For each stimulation site, bars show peak CA1 bursting rates during periods of contingent stimulation (REINFORCEMENT), or noncontingent stimulation (FREE), as a percent of peak rates before conditioning (BASELINE). In the stratum oriens, contingent stimulation produced a significant increase in responding, whereas, equivalent noncontingent stimulation produced a significant decrease. On the other hand, in the molecular layer and Schaffer collaterals, noncontingent stimulation increased rather than decreased CA1 bursting, but the same stimulation delivered on a contingent basis unexpectedly had a lessened excitatory effect. Bars indicate means \pm S.E.M. Numbers in bars indicate number of cells tested. Significantly different from baseline: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (9).

reinforce CA3 cellular activity with dynorphin A_{1-17} . Results from a positive experiment using a pipette concentration of 0.1 mM are shown in Fig. 7 (in dose-finding studies, dynorphin A concentrations of 1 mM—the optimal dose in dopamine and cocaine experiments—caused almost complete suppression of CA3 activity). The frequency of bursts was rapidly increased in two separate periods of operant conditioning; interestingly, the overall firing rate did not exhibit such conditioning and appeared to be uncorrelated with the bursting rate. Table 1 shows averaged dose-response data for dynorphin A in CA3 cells, again indicating significant reinforcement at 0.1 mM. The reinforcing effect of dynorphin A in CA3 cells apparently was blocked in mixtures containing naloxone (1 mM), and no reinforcing effect at the optimal 0.1 mM concentration of dynorphin A was observed in CA1 cells. Thus, preliminary data suggest the possibility of an unexpected double dissociation: CA1 activity may be reinforced with dopamine but not with dynorphin A, whereas, CA3 activity may be reinforced with dynorphin A but not with dopamine.

Delayed Reinforcement in Cellular Operant Conditioning

In behavioral operant conditioning, it is well established that the effectiveness of a reinforcing stimulus is sharply reduced when its presentation following the correct response is delayed (40). Electrical brain stimulation reinforcement, by eliminating the necessity for consummatory responses, permits precise temporal control of the interval between the operant response and reinforcement. By using this method to deliver "primary" reinforcement, and by taking care to minimize possible sources of secondary reinforcement, we found that delays even as short as one second markedly

impede the acquisition of self-stimulation behavior (Fig. 8). Demonstration of a similar delay-of-reinforcement decrement in neuronal operant-conditioning experiments would lend support to the hypothesis that cellular and behavioral reinforcement are interrelated.

Because N-0437 produces reliable baselines of operant conditioning, this compound was used as the reinforcing substance in our initial work on the delay of reinforcement problem (6). The efficacy of cellular operant conditioning associated with reinforcement delays of 0, 100, 200, or 500 msec was determined in an experiment involving 32 CA1 cells; each cell received operant conditioning at a single reinforcement delay. A delay of reinforcement gradient was generated by averaging the peak-bursting rates of the test cells in each delay group (Fig. 9). The curve indicates that reinforcement delays exceeding 200 msec largely eliminate the effectiveness of N-0437 reinforcement in CA1 operant conditioning. The steep gradient of effectiveness of delayed reinforcement makes it unlikely that pharmacological stimulation or some artifact of the injection procedure accounts for the increase in neuronal firing. Rather, the stringent requirement for contingency supports the idea that we have identified a cellular-conditioning process that may play a role in behavioral operant conditioning.

Cellular Operant Conditioning Using Electrical Stimulation of the Brain Slice

Preliminary experiments reveal that electrical brain stimulation may be substituted for locally-applied chemical injections as reinforcement for cellular operant conditioning. The electrical stimulus (ten 0.2-msec rectangular pulses at 100 Hz) was applied directly to the surface of the hippocampal slice as reinforcement for CA1 bursting activity (9). An extracellular micropipette filled with 2 M saline recorded spikes from CA1 cells, and bipolar platinum electrodes, similar to those used in behavioral self-stimulation experiments, were placed about 2 mm from the recording electrode for electrical stimulation reinforcement. Stimulation sites included the molecular layer, stratum radiatum (containing the Schaffer collaterals), and stratum oriens. Current intensities (50–75 μ A) were individually adjusted prior to operant conditioning to a level that did not discharge the recorded cell.

Stratum oriens electrodes unexpectedly produced opposite effects on CA1 activity from those produced by molecular layer or stratum radiatum electrodes (Fig. 10). Both contingent and noncontingent stimulation of the molecular layer or stratum radiatum increased rates of bursting, but noncontingent stimulation caused greater increases than contingent stimulation. (Thus, the excitatory action of molecular layer or stratum radiatum stimulation on CA1 activity may actually be diminished by activity-contingent presentation.) In contrast, noncontingent stimulation of the stratum oriens significantly suppressed CA1 bursting, whereas, the same stimulation delivered on a contingent schedule significantly increased CA1 bursting. This pattern of results, of course, fulfills our criteria for operant conditioning. Indeed, the action of stratum oriens stimulation on CA1 cells closely resembles that produced by reinforcing drug injections; it will be recalled that noncontingent applications of the reinforcing drugs generally were suppressive on CA1 firing. Interestingly, Verney *et al.* (55) report that mesohippocampal dopamine fibers project in the stratum oriens. Hence, it is conceivable that the cellular reinforcing action of stratum oriens stimulation depends at least in part on the activation of these fibers and consequent release of dopamine on CA1 cells.

BEHAVIORAL OPERANT-CONDITIONING STUDIES

In this section, we summarize our initial attempts to test the

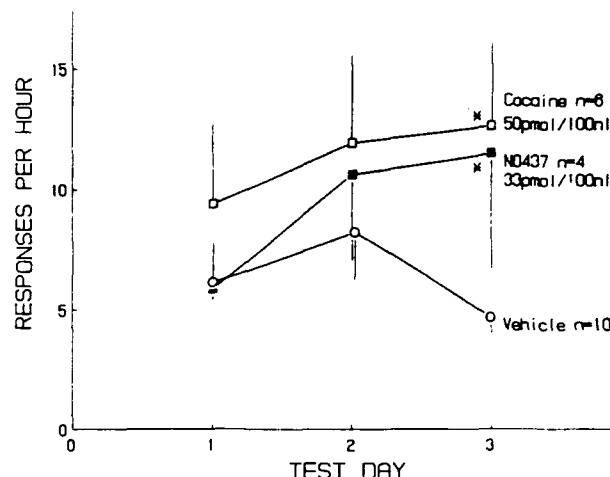


FIG. 11. Self-administration of cocaine and the selective dopamine D2 agonist N-0437 in medial prefrontal cortex. Curves show mean number of self-injections/hr on 3 successive test days for different groups of rats. At least 3 days intervened between tests.

idea that cellular operant-conditioning processes may contribute significantly to behavioral operant conditioning. As already noted, if the two types of operant conditioning were closely related, they might exhibit common properties. Indeed, it might be possible to predict new properties of operant conditioning at one level from previously established properties at the other. Successful prediction of new behavioral data from cellular observations would not only provide evidence of interrelationship, but would help to establish the validity of the cellular operant-conditioning data. In the experiments reported below, previously undescribed behavioral-reinforcing actions of the dopamine D2 agonist N-0437 and the opioid peptide dynorphin A were successfully predicted on the basis of their cellular-reinforcing actions.

Self-Administration of N-0437 in Medial Prefrontal Cortex

To evaluate the reinforcing action of N-0437 at the behavioral levels, we used the cortical self-administration method of Goeders and Smith (23) to determine whether rats would self-administer N-0437 directly in the medial prefrontal cortex. Goeders and Smith (23,24) found that rats will self-administer cocaine in the medial prefrontal cortex (but not in the nucleus accumbens or ventral tegmental area); coinfusion of the selective dopamine D2 receptor antagonist sulpiride attenuated intracortical cocaine self-administration and led to the suggestion that cocaine exerts its reinforcing effect at least in part by an activation of reinforcement-relevant dopamine D2 receptors in medial prefrontal cortex.

Rats were stereotactically implanted with an EMIT guide cannula (Plastic Products Inc.) (12) aimed at the medial prefrontal cortex. N-0437 was dissolved in a Ringer's/alcohol solution, alone and in combination with sulpiride. Approximately 350 μ l of solution were placed in the reservoir of the EMIT delivery system. A lever-press response passed a 250 μ A current across the reservoir electrodes for 5 seconds, evolving hydrogen gas and expelling 100 nl of solution; a holding current maintained the evolved hydrogen in the gaseous state between drug deliveries. Tests were 8 hours in duration, but were terminated earlier to protect against excessive intracranial pressure if the maximum allowable number [40] of daily injections was reached. Rats were



FIG. 12. Photomicrograph of brain section showing placement of EMIT guide cannula just above the CA3 hippocampal field. A sphere of damaged tissue below the tip of the guide cannula marks the site of drug injections. In early tests, this animal exhibited a high rate of self-administration for injections of dynorphin A (1 pmol/100 nl); in later tests, response rates decreased to vehicle levels, possibly as a result of damage to important target sites.

tested every third day.

Acquisition curves for cocaine and N-0437 self-administration are shown in Fig. 11. On the third test day, hourly self-administration rates of cocaine (50 pmol/100 nl) and N-0437 (33 pmol/100 nl) significantly exceeded that of the vehicle control.

Hippocampal Self-Administration of Dynorphin A

As already noted, unanticipated reinforcing actions of dynorphin A on the bursting activity of hippocampal CA3 cells were discovered empirically in neuronal operant-conditioning experiments in the absence of behavioral data. Dynorphins are not

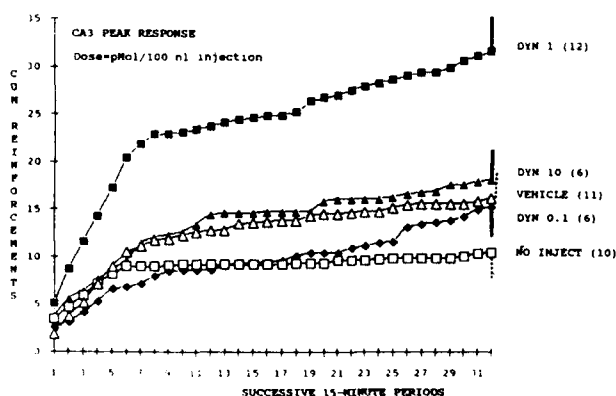


FIG. 13. Cumulative curves of CA3 hippocampal self-administration of different groups of rats given access to various concentrations of dynorphin A (DYN), Ringer's solution (VEHICLE), or to an empty reservoir (NO INJECT). Each rat was tested 3 times. Group averages were calculated by determining the daily peak response for each rat, and then averaging these peak scores over each treatment group. At the optimal dose of 1 pmol/100 nl self-administration of dynorphin A significantly exceeded that of the other concentrations as well as that of the two control groups. Number of rats indicated in parentheses.

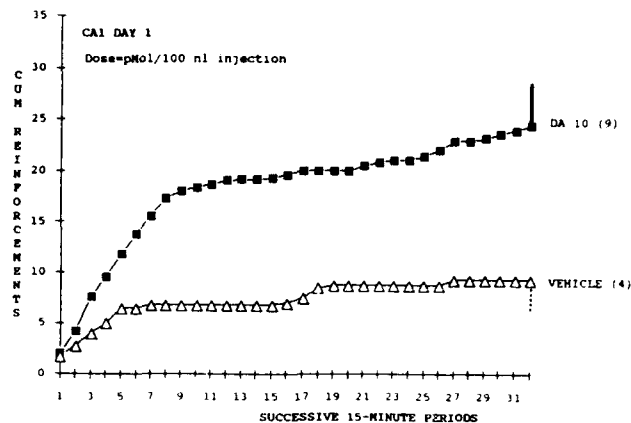


FIG. 14. Cumulative curves of CA1 hippocampal self-administration of dopamine (DA) or the Ringer's solution vehicle. An optimal response to dopamine was obtained on the first test day as shown in this figure. Number of rats per group indicated in parentheses.

thought to be associated with the behavioral reinforcement produced by opiates; indeed, the kappa opiate receptor—for which dynorphin A has high affinity and is surmised to be a natural ligand—is generally assumed to mediate dysphoria rather than euphoria (35). Furthermore, no substance has ever been reported to be self-administered in hippocampus (and perhaps none has ever been tried). Thus, although logically derived from the cellular data, the inference that dynorphin A might exhibit behaviorally-reinforcing properties when injected in the CA3 field of hippocampus seemed quite improbable. This unlikely prediction, nevertheless, seems to have been verified by the results of a hippocampal self-administration experiment in dissertation research performed by Karen Stevens in our laboratory (52,53). Rats were implanted with unilateral EMIT guide cannulas aimed at the CA3 layer of hippocampus (stereotaxic coordinates: 3.8 mm posterior to bregma, 4 mm lateral to midline, 3.1 mm below the surface of the brain) (Fig. 12). Different groups had access to solutions containing different concentrations of dynorphin A, mixtures of dynorphin A and the opiate receptor antagonist naloxone, or the Ringer's solution vehicle. An additional control group (NO INJECT) was tested with the EMIT reservoir empty. One wall of the test chamber contained a 1.5 cm-diameter hole into which the animal could insert its nose. A light over the hole signaled the availability of reinforcing injections. Each nose-poke response activated the EMIT system to deliver a 100-ml injection during a 5-sec infusion interval; a tone was presented throughout the infusion interval. Each drug injection was followed by a 30-sec "timeout" period in which the light was turned off and nose-poke responses had no programmed consequences. Tests were 8 hours in duration, but were terminated earlier (to protect against excessive intracranial pressure and to retard tissue damage) if the maximum allowable number [40] of daily injections was reached. Early termination of test sessions was regularly observed in positive experiments and provided a useful measure of drug-induced reinforcement. Rats were tested every third day for a total of 3 tests.

Rats rapidly learned to self-administer dynorphin A in the CA3 field of hippocampus, often within the first few hours of the first test day. Peak rates of self-administration maintained by each drug concentration are shown in Fig. 13. The reinforcing action of the optimal dose of dynorphin A was partially antagonized in mixtures containing 100 pmol/100 nl naloxone and was completely antagonized in mixtures containing 500 pmol/100 nl naloxone (52). In

related experiments, dopamine was offered to rats implanted with EMIT cannulas in the hippocampal CA1 field. Initial results indicated that dopamine (10 pmol/100 nl) is strongly preferred to vehicle (Fig. 14).

DISCUSSION

The major objective of this research is to identify the functional brain units for positive reinforcement. Does the individual brain cell have the capacity for operant conditioning, or must some larger organization unit—perhaps the substrate of the whole response itself—be identified? And even if the operant conditioning of single units can be demonstrated, does such a cellular process contribute significantly to behavioral operant conditioning?

These questions were evaluated by use of two recently developed methods for the localization of chemical reinforcement effects in the brain. In the first method (cellular operant conditioning), we attempted to reinforce the activity of individual pyramidal cells in hippocampal slices by cellular applications of transmitters or drugs. In the second method, (brain self-administration), we attempted to reinforce a behavioral nose-poke response by injections of the same transmitters or drugs directly into the hippocampus. By concurrent use of both operant-conditioning methods, we attempted to correlate the reinforcing effects of transmitters and drugs at the behavioral and cellular levels.

The cellular observations suggested that the spontaneous bursting of CA1 pyramidal neurons may be reinforced with contingent injections of dopamine and cocaine, whereas CA3 bursting responses may be reinforced with contingently-applied dynorphin A. Using the hippocampal self-administration method, we sought to confirm at the behavioral level this indication of a possible double dissociation between reinforcing agent and hippocampal locus. Preliminary results suggested that dynorphin A is a powerful reinforcer of hippocampal self-administration behavior when injected in the CA3 field; experiments still in progress provide some evidence that dopamine can reinforce self-administration behavior when injected in the CA1 field.

It may be useful to consider alternative explanations of the cellular observations. A major concern would be that dopamine and other putative reinforcing agents may act in the cellular experiments by direct pharmacological stimulation or facilitation of bursting, rather than by some cellular reinforcement process analogous to behavioral operant conditioning. Thus, it could be argued that dopamine might induce at least a minimum level of depolarization that might prevent or delay complete repolarization of a recently-active cell. Such depolarization or delayed repolarization would act in some additive or synergistic way to enhance spontaneous bursting. Thus, the observed facilitation of bursting attributed in our experiments to dopamine-induced reinforcement may actually be due to some direct or indirect excitatory action on hippocampal firing.

This alternative explanation of our results is generally contradicted by the published electrophysiological evidence. In the first place, neurophysiological studies almost without exception report decreased activity of CA1 cells after local microiontophoretic or micropressure application of dopamine [see (8) for review]. More particularly, Benardo and Prince (7) and Gribkoff and Ashe (25) have directly investigated the effects of dopamine on CA1 cell membrane parameters in intracellular studies. Contrary to the suggestion that dopamine may enhance membrane depolarization, both studies show that dopamine actually produces a long-lasting (minutes) membrane hyperpolarization. Furthermore, in neurons that were hyperpolarized by dopamine, spontaneous depolarizations were suppressed "apparently in parallel with the membrane hyperpolarization" (25). (Gribkoff and Ashe (25) also describe a

late-developing depolarizing dopamine action that lasts for hours; however, this late-developing and persistent action cannot explain the contrasting effects of contingent and noncontingent dopamine injections which we observe within the same experimental period of 30–60 minutes.)

A second potential concern is that the brain-slice preparation is insufficiently reduced to demonstrate that individual neurons can be operantly conditioned. Indeed, for many purposes, the value of the hippocampal slice preparation depends precisely on the fact that local circuitry remains largely intact. Furthermore, it is obvious that the extracellular drug treatments are not confined to a single test cell; the microinjections surely must affect a small subset of neighboring pyramidal cells and interneurons as well as presynaptic fibers and terminals from projection systems. Although these points are valid, such criticism, nevertheless, fails to take into account the absolute requirement for response-reinforcement contingency in the demonstration of operant conditioning. Our results indicated that cellular operant conditioning depends critically on the close contingency of neuronal firing and the microinjected drug reinforcement: chemical reinforcements delayed more than 200 msec are essentially ineffective. Since reinforcement is delivered only when the recorded cell fires, it is clear that all neighboring cells which do not fire synchronously with that cell cannot receive activity-contingent drug reinforcement. What is left then as a possible unit for reinforcement is either the single pyramidal cell itself (and its synapses), or a very small ensemble of synchronously-firing neighbors. Such ensembles would have to be distributed essentially ubiquitously in the CA1 and CA3 layers in order to account for all of our positive experiments. These are remote assumptions, but in any case the hypothetical ensemble should behave in many ways as a multiple version of the single cell. Hence, as a first approximation, the cell and the ensemble may be treated as logical equivalents until the question of their differentiation becomes an issue.

The validity of cellular bursting as the dependent variable in the proposed experiments also may be questioned. As noted, an arbitrary decision was made initially in choosing which aspect of unit activity to reinforce. In behavioral-conditioning experiments, however, it is explicitly understood that the operant response is selected arbitrarily; the pertinent question is whether the probability of that arbitrary response can in fact be increased by reinforcement. We believe this pragmatic test of suitability has been satisfied in the case of the bursting response. Furthermore, we find that the bursting of hippocampal cells has attractive possibilities as a reinforceable cellular response, in view of the report (30) that such bursting is associated with substantial increases in intracellular calcium. As suggested by Turner *et al.* (54) and Lynch *et al.* (33), calcium influx is likely to be the trigger for the long-term changes in cellular excitability that underlie long-term potentiation (LTP). Moreover, Kandel (27) has suggested that calcium influx may serve as the ionic marker of recent activity for activity-dependent presynaptic facilitation in cellular classical conditioning. In turn, we have speculated along similar lines that calcium influx may prime the bursting hippocampal cell for chemical reinforcement (45,50).

The present emphasis on the hippocampus as a potential substrate for operant conditioning also may be questioned on the grounds that this site is not commonly associated with natural or drug-induced reinforcement. Although sometimes reported as providing mild reinforcement, electrical stimulation of hippocampal sites does not support high or even stable rates of self-stimulation [see (52) for review]; and, as already noted, the hippocampus seems to have been ignored as a brain site for chemical self-administration studies. Our response to this objection is largely pragmatic: experiments that make use of hippocampal locations for cellular and behavioral operant conditioning seem

to work well.

Finally, it may be asked whether the cellular properties observed in slices are the same as those observed in an intact brain. Specifically, is it appropriate to assume that the cellular operant conditioning observed in vitro in slices is reflective of a natural hippocampal cellular operant-conditioning process in vivo? Preliminary data from cortical units obtained in vivo (9) suggests similarities between in vivo and in vitro cellular operant conditioning, but nevertheless, the in vitro studies must be interpreted with considerable caution. The importance of performing parallel behavioral experiments in attempts to validate the cellular data by correlation and prediction is again emphasized.

In summary, we conclude that our observations are consistent with the possibility that the activity of individual neurons may be operantly conditioned by direct cellular applications of reinforcing

transmitters or drugs. If so, and since it seems unlikely that a brain cell would display a gratuitous capacity for operant conditioning, the individual neuron could be an important functional unit for positive reinforcement in the brain. Successful prediction of new behavioral data (e.g., hippocampal self-administration of dynorphin A) from prior cellular observations not only helps to validate the cellular data, but provides suggestive evidence of interrelationship between cellular and behavioral operant-conditioning processes.

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REINFORCEMENT OF HIPPOCAMPAL BURST ACTIVITY BY THE
CANNABINOID RECEPTOR AGONIST CP-55,940

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ABSTRACT

Involvement of cannabinoid receptors in behavioral reinforcement has been demonstrated by self-administration of Δ^9 -tetrahydrocannabinol (THC) and THC-induced reduction of self-stimulation reward thresholds. Cannabinoid receptors are found in high density in rat hippocampus and other brain areas. Using the hippocampal-slice preparation, we attempted to demonstrate *in vitro* operant conditioning of the spontaneous bursting of CA1 neurons using local micropressure applications of the high-affinity synthetic cannabinoid receptor agonist CP-55,940 as reinforcement. Approximately 60% of the tested neurons showed increased burst activity after a series of brief, burst-contingent applications of CP-55,940 at pipette concentrations of 5 and 10 μ M. Identical microinjections of CP-55,940 administered independently of cellular activity did not increase, and in fact suppressed, the rate of bursting. Since direct stimulating effects of CP-55,940 on CA1 activity can thus be ruled out, we conclude that burst-contingent cannabinoid receptor activation can reinforce hippocampal firing.

Key Words: Δ^9 -tetrahydrocannabinol, CP-55,940, Reinforcement, Operant Conditioning, Hippocampal CA1 cells, Extracellular Recording, Marijuana, Cannabinoid Receptors.

INTRODUCTION

The widespread use of marihuana in many societies, and the consistent reports of euphoria associated with this drug, suggest that this substance has reinforcing properties (5). However, with few exceptions, laboratory studies of cannabinoids in animals reveal weak and inconsistent reinforcing effects, possibly due in part to the extreme insolubility of these drugs in water. Nevertheless, involvement of cannabinoids in behavioral reinforcement has been demonstrated by self-administration of Δ^9 -tetrahydrocannabinol (THC) (14, 15) and by THC-induced reduction of self-stimulation reward thresholds (2, 3). Recently, cannabinoid receptors characterized and precisely localized by the highly potent synthetic cannabinoid agonist [3 H]-CP-55,940, have been found in great abundance in the rat basal ganglia, cerebellum, olfactory bulb and hippocampus (4, 8).

Apparent "operant conditioning" of neuronal firing has been demonstrated following burst-contingent applications of dopaminergic and opioid drugs to individual cells in hippocampal slices (1, 13). The present study was undertaken to determine whether CP-55,940 would show similar reinforcing actions when applied to individual hippocampal CA1 cells contingent on burst activity. Noncontingent applications of the same doses of CP-55,940 provided a control for direct pharmacological stimulation or facilitation of bursting, although generally THC is reported to inhibit CA1 activity (10, 16). The present finding that cannabinoid receptor activation readily reinforces spontaneous CA1 bursting activity provides support for the idea that our *in vitro* ^{neuronal} ~~cellular~~ operant conditioning paradigm represents a cellular analog of behavioral operant conditioning.

METHODS AND MATERIAL

The experiments were performed on transverse hippocampal slices prepared from male Sprague-Dawley rats (200-270 g). The rats were lightly anesthetized with Halothane and decapitated. The brain was removed rapidly from the skull and allowed to cool at 4 °C in artificial cerebrospinal fluid (ACSF) containing NaCl (124 mM), KCl (5 mM), CaCl₂ (2.4 mM), MgSO₄ (2 mM), KH₂PO₄ (1.25 mM), NaHCO₃ (26 mM) and glucose (10 mM). The hippocampus was dissected out and sliced into 400- μ M slices using a McIlwain tissue chopper. Using an eyedropper, 6-8 slices were individually transferred to a static chamber where they were supported on nylon mesh at the surface of the ACSF solution in an oxygenated atmosphere (95% O₂, 5% CO₂) at 35 °C. The ACSF solution in the static chamber was changed every 30 min, unless prohibited by potential disruption of an ongoing experiment. Following incubation for at least 2 hr, cellular activity was recorded using single-barrelled extracellular micropipettes filled with vehicle or drug solution and with the tip broken to permit pressure ejection of a 10 μ -diameter droplet following a 50-msec application of nitrogen at 15 P.S.I. During operant conditioning, micropressure injections of drug were applied directly to the cell for 50 msec following bursts of activity. Drug-induced increases in bursting are necessary but not sufficient evidence of cellular operant conditioning, since the drug treatments may directly stimulate or facilitate cellular firing. As a mandatory control for such pharmacological stimulation, the same drug injections must be administered independently of bursting on a noncontingent or random basis. Cellular reinforcing effects may be inferred only if the noncontingent injections are relatively ineffective. The experimental setup is shown diagrammatically in Fig 1. A burst was defined as a train of firing containing N or more spikes with a maximum interspike interval (ISI) of t msec. Normally, reinforceable bursts of activity contained

3-6 spikes with a maximum ISI of 10 msec. The parameters were set individually for each test neuron such that bursts occurred at a baseline rate of approximately 5 per min.

A complete neuronal operant conditioning experiment involved six stages: *Baseline*: the rate of bursting prior to operant conditioning was determined in a baseline period of approximately 5-10 minutes. *Reinforcement*: each burst was now followed by an injection of the test solution. To minimize injection artifacts, neuronal activity during and for 3 sec after each injection was excluded from analysis and had no programmed consequences. *Extinction*: reinforcement was terminated and recording continued until the baseline burst rate was recovered. *Matched (Free) Injections*: noncontingent injections of the test solution were given at regular intervals to determine the direct pharmacological effects on neuronal activity. The number of injections was matched to the 3-5 highest injection rates received during the prior reinforcement period. Again, neuronal activity during and for 3 sec after each injection was excluded from analysis. *Washout*: a second baseline period was given in order to allow residual effects of drug administration to dissipate and for baseline burst rates to return. *Reacquisition*: a second period of reinforcement was scheduled, whenever possible, in order to compare rates of original acquisition and reacquisition and to ascertain the viability of the preparation following noncontingent injections.

A stock solution of CP-55,940 at 10 mM in absolute ethanol was stored at -20 °C. Test solutions were prepared by diluting the stock solution with saline to final CP-55,940 concentrations of 2.5, 5, 10 and 100 μ M. Vehicle-control tests were performed with saline containing the same concentration of ethanol (0.1%) as the optimally-reinforcing 10 μ M CP-55,940 solution.

RESULTS

The firing of hippocampal CA1 cells was selectively increased by brief, burst-contingent injections of CP-55,940 in a dose-dependent manner (Fig. 2). Approximately 60% of the neurons tested were successfully reinforced by contingent application of CP-55,940 at pipette concentrations of 5 and 10 μ M; at concentrations of 2.5 and 100 μ M, there was only a single positive experiment (Table 1). Results from a representative experiment with the 10- μ M dose of CP-55,940 are depicted in Figure 3. In two separate periods of operant conditioning (REINF), the frequency of bursts and the overall firing rate were gradually increased after several burst-contingent applications of CP-55,940. The same CP-55,940 injections administered noncontingently (MATCH) did not increase, and in fact rapidly suppressed burst activity, while the overall firing rate was unaffected. Because burst activity was suppressed by these noncontingent administrations, direct stimulant or facilitating effects of CP-55,940 on neuronal activity may be ruled out as an explanation of the increased bursting observed in reinforcement periods.

In a second set of experiments, we reversed the order of treatments and administered the noncontingent applications of CP-55,940 (5 and 10 μ M) before we tested for operant conditioning with contingent injections. This control was instituted in order to ensure that deterioration of the preparation (after prolonged testing and repeated injections) did not obscure potential facilitatory effects of the noncontingent applications in the first set of experiments. An example of such an experiment is shown Fig. 4. After a few minutes of baseline recording, CP-55,940 (5 μ M) was initially applied noncontingently (FREE) at a rate of 10 injections/min. In the case of this unit, burst rate and overall spike frequency were almost immediately and totally inhibited by the noncontingent injections. However, and despite this total inhibition of cellular activity, burst-contingent applications of CP-55,940 during the subsequent reinforcement period (REINF) caused

a marked increase in bursting. In similar experiments in which the 10 μ M-concentration of CP-55,940 was used, the initial series of noncontingent injections caused, in 6 out of 7 cases, such a profound and long-lasting inhibition of cellular firing that baseline activity could not be recovered and testing with contingent injections could not be performed. Table 2 summarizes the results of 28 experiments--at the 5- μ M concentration of CP-55,940--in which the two treatment sequences were compared. There was little relationship between the order of treatments and the experimental outcome; similar increases in bursting were produced by contingent injections of CP-55,940, whether they preceded or followed the series of noncontingent injections. Similarly, noncontingent injections of CP-55,940 suppressed CA1 bursting activity to approximately the same degree, whether they preceded or followed the contingent injections.

It should also be noted in Figs. 3 and 4 that the bursting rate turned down at the end of all reinforcement periods. This effect typically is observed when high rates of bursting have been generated by the reinforcement procedure, and we attribute it to an unsurmountable inhibitory effect of CP-55,940 on cellular firing when the injection rate (and therefore the local CP-55,940 concentration) is excessive. The failure of the very high (100- μ M) concentration of CP-55,940 to produce operant conditioning may have a similar explanation. In an effort to protect the unit from excessive CP-55,940 concentrations, we typically terminated the reinforcement period at the point that the acquisition curve turned down. In this regard, mention should be made of the fact that relatively high concentrations (5-10 μ M) of CP-55,940 were required for reinforcement. However, ^{effective} total drug dose is determined not only by the concentration of the solution in the micropipette, but also by other parameters, such as injection duration and volume. Because drug injections in this experiment had to be delivered in close contingency to ^{unpredictable} bursts of cellular activity, it was necessary to use an exceedingly short injection duration (50 ms) and small volumes (approximately 5 picoliters). After diffusion to action sites, these minute droplets of drug are presumably diluted to

concentrations comparable to those produced in more conventional neuropharmacological or biochemical studies, where lower initial concentrations are applied in greater volumes for much longer durations.

DISCUSSION

The present findings provide the first demonstration of reinforcing activity associated with the synthetic cannabinoid receptor agonist CP-55,940. Burst-contingent applications of CP-55,940 increased the rate of hippocampal CA1 bursting, whereas the same drug injections, applied independently of cellular activity, suppressed bursting. Such opposite actions of contingent and noncontingent drug applications on CA1 bursting have previously been observed in similar experiments with cocaine, dopamine, and dopamine D₂ agonists (1, 13). The direct inhibitory actions of noncontingent injections rule out direct stimulation as an explanation of the rate-increasing action of contingent injections, and thus support the interpretation that contingent injections act by a cellular mechanism analogous to behavioral reinforcement. Indeed, burst-contingent administrations of inhibitory agents, such as CP-55,940, must overcome their own immediate inhibitory actions in order to display their longer-lasting (second-messenger mediated?) facilitatory reinforcing effects.

The cellular reinforcing activity of CP-55,940 is in agreement with previous reports demonstrating behavioral reinforcement with natural cannabinoids, *viz.*, self-administration of THC and THC-induced reduction of self-stimulation reward thresholds (2). Van Ree *et al.* reported self-administration of THC, but the reliability and intensity of this effect was not impressive and the percentage of rats that initiated and maintained self-administration was only 40% (15). On the other hand, THC, at a dose estimated to be pharmacologically relevant to moderate human use of marijuana, clearly enhanced medial forebrain bundle electrical brain stimulation reinforcement and also enhanced basal and stimulated dopamine release in reward-related brain regions, such as nucleus accumbens (9). In a recent review of these data, Gardner and Lowinson (3) suggest that marijuana's actions on brain reward systems is fundamentally similar to that of other drugs of abuse.

Substantial evidence suggests that the endogenous reward system is made up of, or is regulated by, dopamine- and opioid peptide-containing neurons (12, 17). The recent availability of subtype-selective ligands has allowed detailed examination of the role of various dopamine and opioid receptors in the mediation of natural and drug-induced reward. The reinforcing effects of stimulant drugs of abuse apparently involve both D_1 and D_2 receptors, whereas μ and δ receptors appear to mediate the reinforcing effects of opioids (11). At the second messenger level, the reward-related dopamine D_2 and μ and δ opioid receptors share the ability to couple G_i proteins that mediate inhibition of adenylate cyclase (11). Similarly, the cannabinoid receptor has been found to interact with G_i proteins to inhibit adenylate cyclase (6, 7). A common signal transduction mechanism, inhibition of adenylate cyclase via G_i proteins, could thus be generally implicated in the reinforcing properties of dopaminergic, opioid and cannabinoid drugs of abuse.

In summary, the present studies indicate that cannabinoid receptor activation, like dopamine receptor activation, can reinforce hippocampal CA1 burst activity. Cannabinoid receptors, like dopamine and opioid receptors, may play an important role in both cellular and behavioral operant conditioning.

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FIGURE CAPTIONS

Fig. 1. Schematic diagram of cellular operant-conditioning experiment. A single-barrelled glass micropipette for simultaneous extracellular recording and pressure injection is filled with CP-55,940 or vehicle and aimed at spontaneously active hippocampal cells in the pyramidal cell layer of CA1. Amplified action potentials are displayed on a digital oscilloscope and are processed through a spike enhancer and window discriminator to increase the signal-to-noise ratio and to isolate signals when multiple-unit activity is encountered. When the computer recognizes a reinforceable burst of activity (based on criteria established individually for each test neuron before operant conditioning), the pressure-injection pump is activated for 50 msec to deliver an approximately 10 μ -diameter droplet of drug in the close vicinity of the cell. Oscilloscope display shows (upper trace) a burst of firing recorded extracellularly from a CA1 cell, exhibiting typical decrescendo pattern with progressively smaller spikes occurring later in the burst. The lower trace shows 1-msec rectangular pulses indicating each spike counted by the computer, and the initial part of the 50-ms rectangular pulse signalling the operation of the pressure-injection pump and the delivery of the drug reinforcement.

Fig. 2. Maximal reinforcing effects of various doses of CP-55,940 on CA1 bursting. Bars show peak rates of bursting at each dose, calculated by averaging the two highest 50-sec bursting scores recorded from each unit during reinforcement periods and then averaging for the treatment group. Neurons reinforced with 10 μ M CP-55,940 exhibited significantly higher rates of bursting than did controls reinforced with the 0.1% alcohol vehicle (between-groups comparison). Symbols over bars show significant differences from peak baseline scores (within-groups comparison): * $P < 0.05$; ** $P < 0.01$.

Fig. 3. Operant conditioning of the spontaneous bursting activity of a CA1 hippocampal cell with local injections of CP-55,940 used as reinforcement. The activity of the unit throughout seven phases of a complete experiment is shown. Each point on the abscissa shows the number of bursts (lower graph) and the number of spikes (upper graph) in successive 50-sec recording intervals. Prior to the first baseline phase, burst criteria of 3 or more spikes with a maximum interspike interval of 10 ms were selected. These criteria gave a burst rate for this unit that never exceeded 7 per 50 sec in the initial baseline period (BASE). In the reinforcement period (REINF), CP-55,940 (10 μ M in 165 mM saline containing 0.1% ethanol) was applied for 50 ms immediately after each burst. Following a second baseline period, the same CP-55,940 injections were delivered (MATCH) independently of the unit's behavior as a control for possible stimulant effects. The number of injections was matched to those earned in the 3 highest periods of the reinforcement phase. Rates of bursting and overall firing were increased by the contingent CP-55,940 injections during the reinforcement periods, but were decreased when the same injections were administered noncontingently in the matched-injection period. Positive results in the second reinforcement period demonstrated that the unit remained viable throughout the experiment.

Fig. 4. Operant conditioning of hippocampal CA1 bursting with CP-55,940 reinforcement. This experiment is similar to that shown in Fig. 3, except that the sequence of the noncontingent and contingent injections was reversed. For details, see text and Fig. 3.

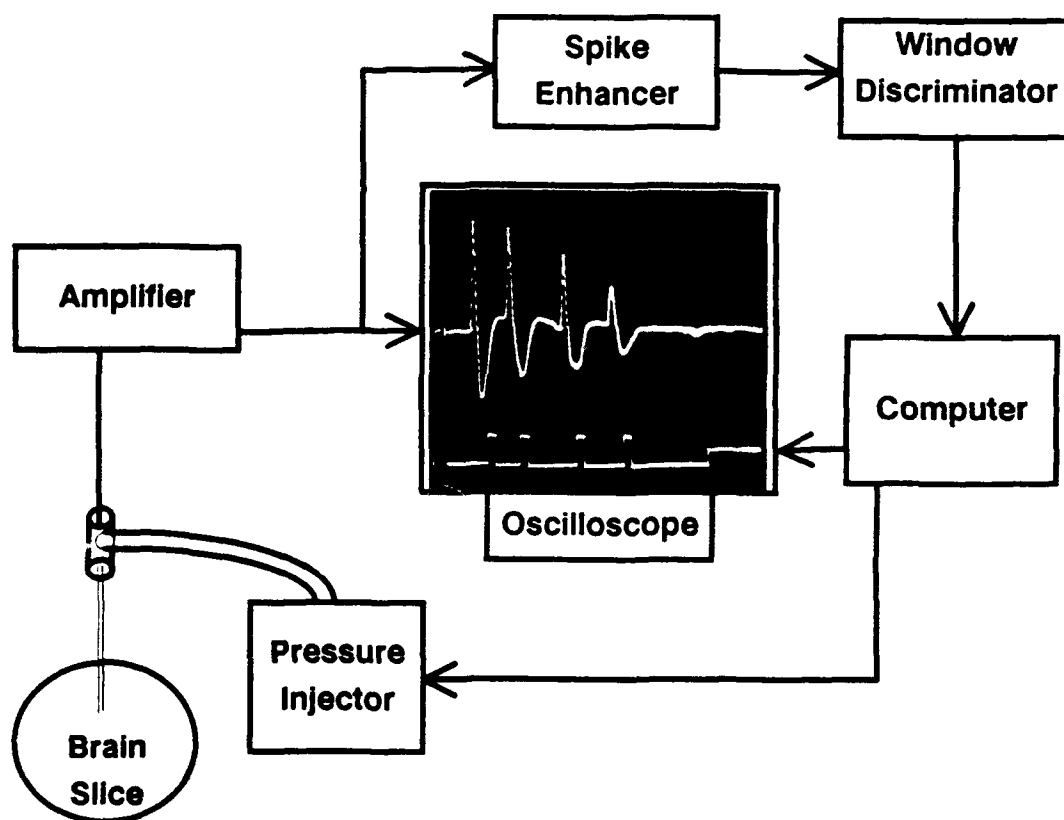


Fig 1

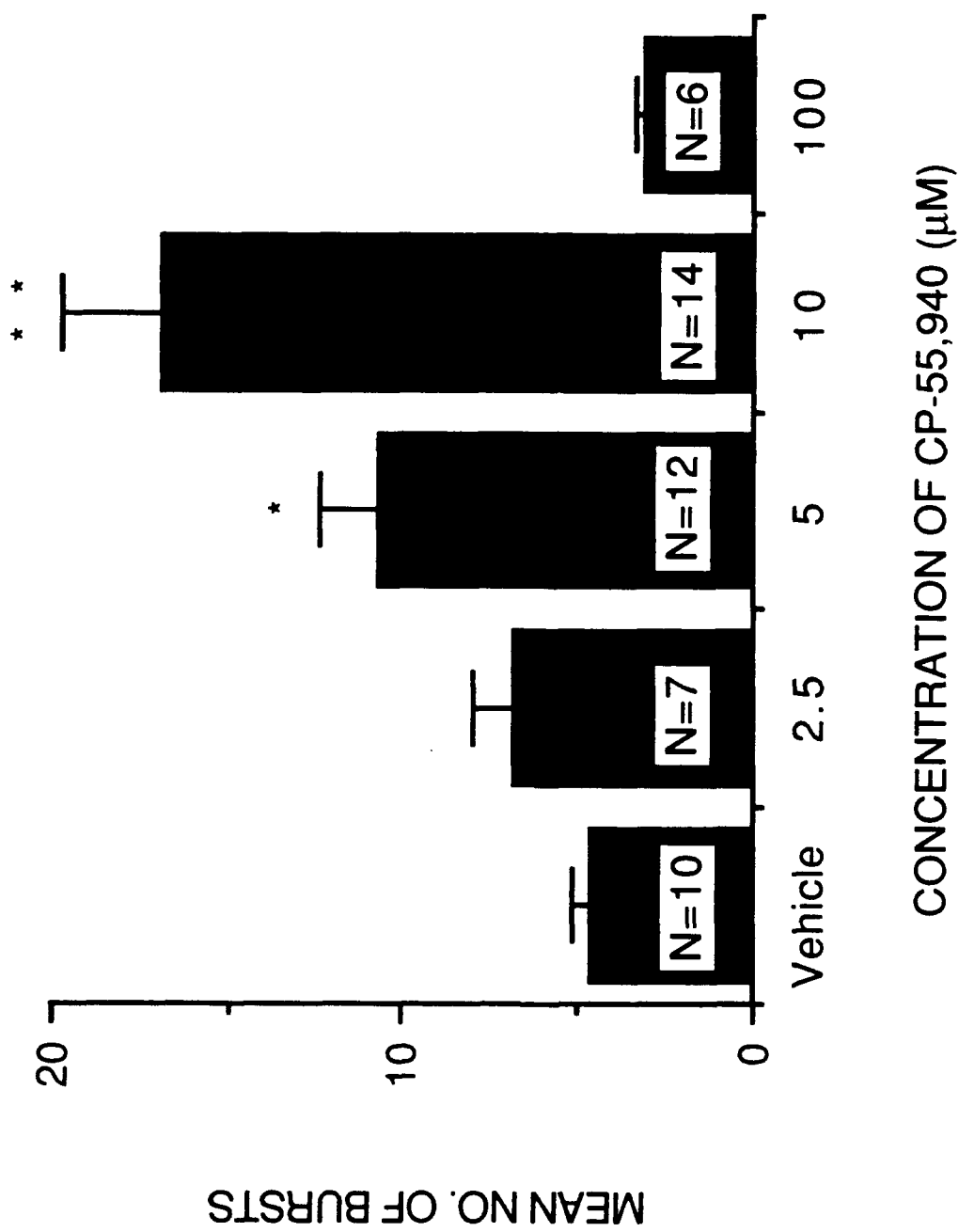
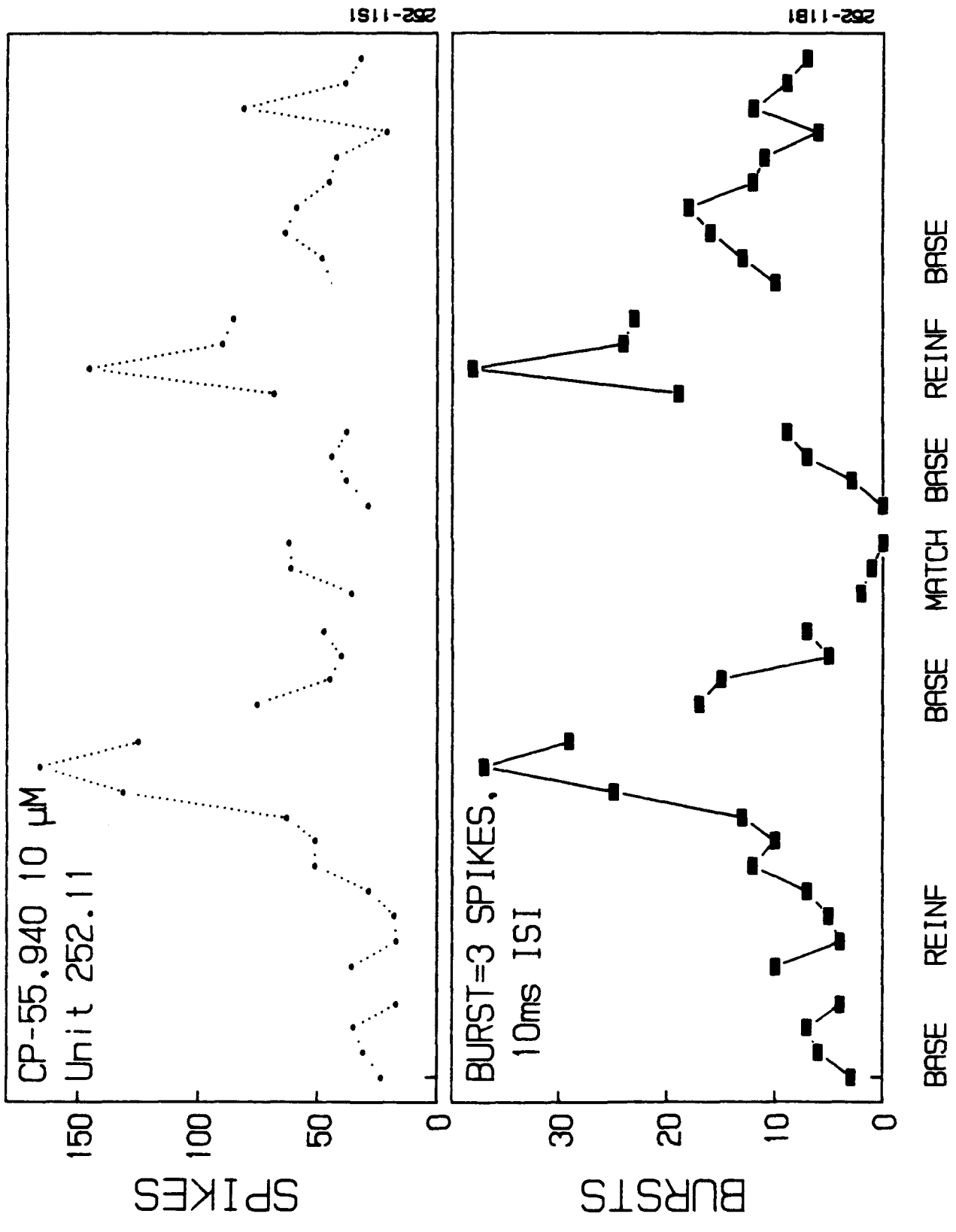
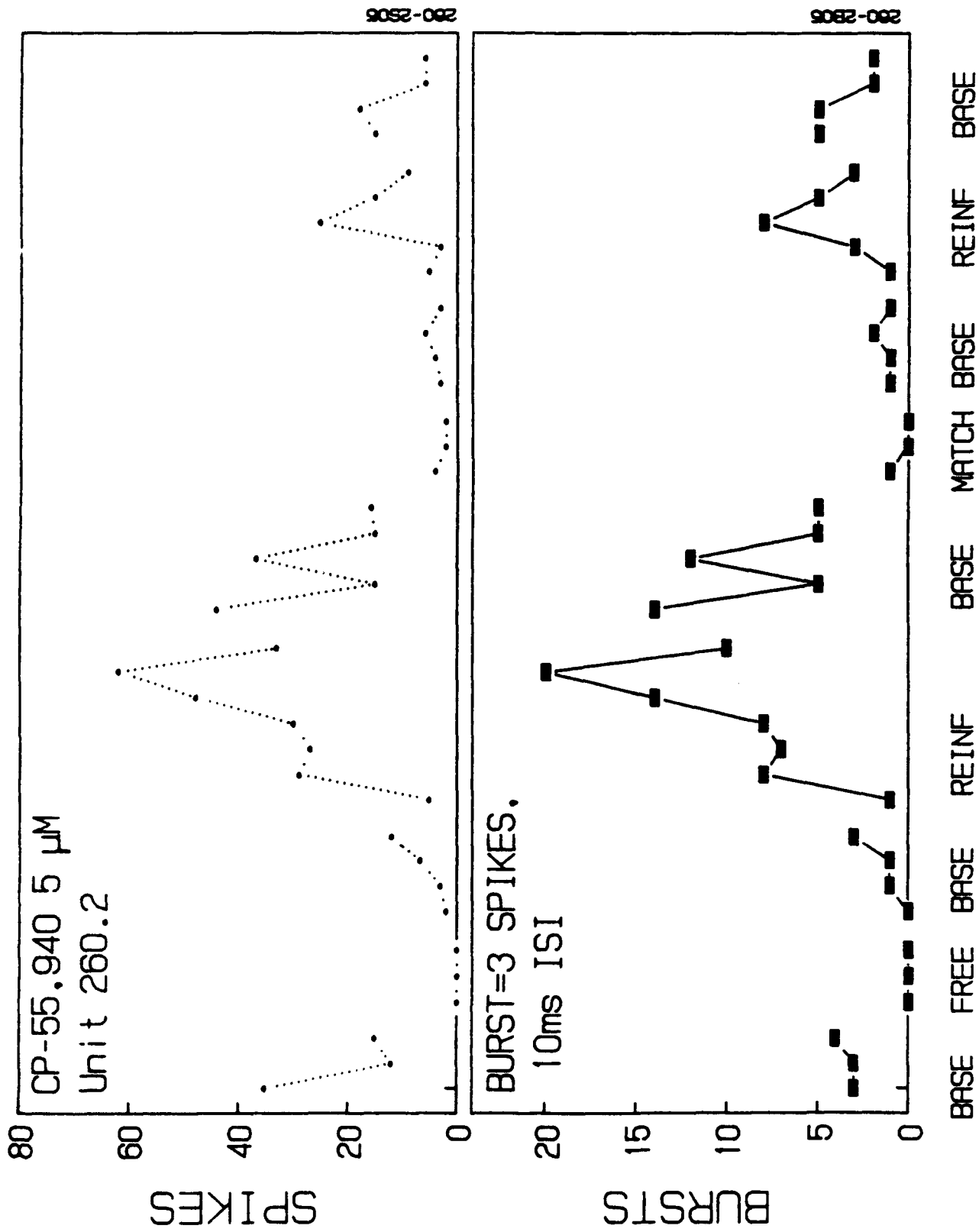


Fig 2

SUCCESSIVE 1-MIN SAMPLES





SUCCESSIVE 1-MIN SAMPLES

Table 1. Probability of successful operant conditioning as a function of dose of CP-55,940.

| Dose of CP-55,940 (μ M) | No. of Units Tested N | No. of Positive Experiments ⁺ N (%) | No. of Negative Experiments* N (%) |
|------------------------------|--------------------------|---|---------------------------------------|
| Vehicle | 9 | 0 (0) | 9 (100) |
| 2.5 | 7 | 1 (14) | 6 (86) |
| 5 | 12 | 7 (58) | 5 (42) |
| 10 | 17 | 10 (59) | 7 (41) |
| 100 | 6 | 0 (0) | 6 (100) |

⁺ Positive experiments are those in which the probability of bursting progressively increased following contingent injections of CP-55,940 and did not increase, or decreased, following non-contingent injections.

* Negative experiments are those in which bursting was not increased following contingent injections of CP-55,940.

Table 2. Effect of treatment sequence on CP-55,940 (5 μ M)-reinforced CA1 operant conditioning.

| Sequence | No. of Units Tested N | No. of Positive Experiments* N (%) | No. of Negative Experiments** N (%) | Peak Bursting Rates | | |
|---------------------------------|--------------------------|---------------------------------------|--|---------------------|------------------------------|-----------------------------|
| | | | | Baseline | Contingent | Non-contingent |
| Contingent Injections First | 12 | 7 (58) | 5 (42) | 3.2 \pm 0.3 | 10.4 \pm 1.7 ⁺⁺ | 1.8 \pm 0.7 |
| Non-Contingent Injections First | 16 | 7 (43) | 9 (57) | 4.2 \pm 0.5 | 9.6 \pm 2.2 ⁺ | 1.2 \pm 0.4 ⁺⁺ |

* Positive experiments are those in which the probability of bursting progressively increased following contingent injections of CP-55,940 and did not increase, or decreased, following non-contingent injections.

** Negative experiments are those in which bursting was not increased following contingent injections of CP-55,940.

+ Significantly different from baseline, $p < 0.05$; ++ $p < 0.01$.

Receptor Subtypes in Opioid and Stimulant Reward

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Abstract: Studies of the behaviourally-reinforcing actions of opioid and stimulant drugs of abuse are reviewed in an attempt to identify their reward-related brain receptors. We focus on data generated by drug self-administration, brain stimulation reinforcement, and conditioned place preference paradigms. A consistent body of evidence supports a role for μ and δ , but not κ , receptors in opioid reward. Stimulant reward apparently involves both D_1 and D_2 receptors; the data favour D_2 mediation of stimulant drug reinforcement with a permissive or modulatory role for D_1 receptors. The reward-relevant opioid and dopamine receptors, as well as the cannabinoid (marijuana) receptor, share the ability to couple G_i proteins that mediate inhibition of adenylate cyclase and stimulation of K^+ conductance. These signal transduction mechanisms thus may be generally implicated in the reinforcing properties of diverse drugs of abuse.

Opioid drugs and psychomotor stimulants can be conceptualized as pharmacological reinforcers (rewards), whose addictive or behaviourally-reinforcing actions are mediated via the brain's natural reward systems. Much evidence suggests that the endogenous reward system is made up of, or is regulated by, dopamine- and opioid peptide-containing neurones (Stein 1978; Wise 1978 & 1987). The recent availability of subtype-selective ligands has allowed behavioural pharmacologists to examine the role of various opioid and dopamine receptors in the mediation of natural and drug-induced reward. This evidence, and some implications for biochemical hypotheses of opioid and stimulant reinforcement, are reviewed here.

We focus on data generated by the three principal behavioural methods used to measure drug-induced reward in animals: viz., drug self-administration, brain stimulation reinforcement (BSR) and conditioned place preference. In spite of the complexities of the reinforcement problem, these methods have provided a reasonably consistent picture of the particular dopamine and opioid receptors that may be involved in the rewarding action of psychomotor stimulants and opioids. As we shall see, these reward-relevant receptors as well as some others (e.g., the cannabinoid receptor) share similar signal transduction mechanisms and have in common the ability to interact with G_i proteins that inhibit adenylate cyclase. These common mechanisms thus could mediate the reinforcing actions of many abused drugs.

Opioid receptors

Three major opioid receptors are currently recognized, each characterized by a distinctive brain distribution and pharmacological profile: μ , which are selectively activated by morphine, δ , by met- and leu-enkephalin, and κ by benzomorphan opioids.

Opioid self-administration. Animals will learn to perform an arbitrary response, such as pressing a bar, in order to receive an intravenous drug injection. The drug injection

is said to be reinforcing if response rates increase (acquisition) and remain elevated (maintenance) when compared to undrugged controls given access to vehicle solutions, or to "yoked" controls that receive drug injections simultaneously with reinforced animals, but independently of their own lever-pressing behaviour. As the opioid dose is increased, the response rate first increases and then decreases, resulting in an "inverted-U" dose-response curve (Pickens *et al.* 1978). Hence, a rate-maximizing intermediate dose level can be identified about which both higher and lower doses will reduce response rates. Higher drug doses have prolonged actions and decrease response rates by increasing the interval between self-injections. Lower doses are presumed to reduce response rates by lessening the reinforcing efficacy of the injections; if the dose falls below the reinforcement threshold, self-administration will be extinguished. Pretreatment with reinforcing agonists usually has the same rate-reducing effect as increasing the self-administered dose—in effect, such pretreatment shifts the descending limb of the dose-effect curve to the left. Conversely, competitive antagonists cause compensatory increases in responding by rightward displacement of the dose-effect curve; however, very high doses of antagonists may block self-administration altogether and produce extinction-like behaviour.

The involvement of the μ receptor in opioid reward is immediately suggested by the fact that the prototypical opioid reinforcer morphine interacts almost exclusively with the μ receptor (Kosterlitz 1987). This suggestion of μ involvement is supported by a number of rhesus monkey self-administration studies. Thus, the relative potency of several opioids for maintenance of maximum self-administration rates correlated well with Martin's criteria for μ agonist activity in the spinal dog (Young *et al.* 1981). More recently, the ability of quada-zocine (a non-selective opioid antagonist) to cause compensatory increases in the self-administration of alfentanil (μ -preferring agonist) was subjected to *in vivo* pA₂ analysis (Bertalmio & Woods 1989). The apparent

pA₂ of 7.6 was similar to values obtained for quadazocine against various μ agonists in other behavioural paradigms (e.g., analgesia), but distinct from values obtained against κ agonists (5.7 to 6.4). It was concluded that quadazocine antagonism of μ , but not κ , receptors, blocked alfentanil reinforcement. Similarly, the potent κ -preferring agonist ethylketocyclazocine proved unable to maintain self-administration at rates above controls following substitution for codeine (Woods *et al.* 1979). In contrast to these negative findings in codeine-habituated monkeys, both acquisition and maintenance of ethylketocyclazocine self-administration has been demonstrated in drug-naïve rats, albeit over a narrow dose range and at lower maximal injection rates than with morphine (Collins *et al.* 1984; Tang & Collins 1985). These moderate reinforcing effects of ethylketocyclazocine may not be κ -mediated however, but rather could arise from the drug's known partial agonist activity at μ receptors (Pasternak 1980; Ward & Takemori 1983). Consistent with this suggestion, rats did not acquire self-administration behaviour when offered the highly selective κ agonist, U50,488 (Tang & Collins 1985).

Experiments involving opioid agonist pretreatment also implicate the μ receptor in opioid self-administration. As noted, reinforcing agonists can cause compensatory decreases in self-administration rates by effectively shifting the inverted U-shaped dose-effect curve to the left. This approach has been used to study the effect of supplemental infusions of μ -preferring fentanyl or κ -selective U50,488 on heroin self-administration in rats (Koob *et al.* 1986). A single injection of fentanyl prolonged the time interval between responses for heroin in dose-dependent fashion, as did supplemental injections of heroin itself; this suggests that fentanyl and heroin may activate the same opioid receptor type (i.e., μ) to produce reward. U50,488 failed to cause reward-indicating increases in the interinfusion interval. Furthermore, extinction-like responding was seen after substitution of U50,488 for heroin, again indicating that U50,488 itself was not reinforcing. Fentanyl, as expected, was readily self-administered in substitution experiments. All of these results implicate μ , but not κ , opioid receptors in heroin reinforcement.

The development of intracranial self-administration techniques has enabled researchers to tentatively identify brain loci that may mediate the reinforcing effects of abused drugs. Such investigations have revealed several brain regions that will support the intracranial self-administration of opioids (e.g., ventral tegmental area, Bozarth & Wise 1981; nucleus accumbens, Olds 1982; Goeders *et al.* 1984; hippocampus, Stevens *et al.* 1991). Most of the opioid agonists used were μ or δ -preferring (morphine or met-enkephalin), but naloxone-reversible self-administration also has been obtained with low concentrations of κ -preferring dynorphin A (1-17) (Stevens *et al.* 1991). Because this peptide has marked affinity for μ and δ as well as κ receptors, antagonists more selective than naloxone at each of the three opioid receptors were tested. Neither κ nor δ antagonism affected dynorphin self-administration, but μ

antagonism completely blocked it. It was concluded that, at least in the hippocampus, dynorphin's reinforcing actions are mediated at μ receptors.

Opioid receptors in brain stimulation reward (BSR). Animals will readily learn a response such as pressing a lever in order to receive electrical stimulation of discrete brain regions. It is generally assumed that such self-stimulation behaviour is rewarded by the electrically-induced release of reinforcing neurotransmitters. Since the stimulating electrode undoubtedly causes the simultaneous release of many transmitters, pharmacological antagonists are used to assess which of these are associated with reinforcement. It is important to discriminate drug-induced reward blockade from non-specific impairments of motor performance or alertness. One approach is to examine the patterns of behavioural change induced by the drug. Thus, if a antagonist produces a pattern of gradual, within-session response decrement (like that observed in extinction), then reward-related receptor blockade is usually inferred. In another paradigm, response-rate data are collected at different stimulation frequencies or intensities. The resulting stimulus-response curve resembles a pharmacological dose-response curve in that, without a change in maximal rates, competitive antagonists can cause rightward displacement of the curve and agonists can cause leftward displacement. Finally, changes in self-stimulation reinforcement thresholds, which can be measured independently of response rates, can serve as a useful indicator of a drug's reinforcing effects. Both stimulant and opioid drugs of abuse have been found to be particularly effective in lowering reinforcement thresholds (Stein & Ray 1960; Kornetsky *et al.* 1979).

μ -Preferring opioid agonists reduce brain stimulation reward thresholds following peripheral administration (e.g., Lorens & Mitchell 1973; Marcus & Kornetsky 1974). The κ -preferring agonist ethylketocyclazocine had no effect on hypothalamic reward thresholds (Unterwald *et al.* 1987), but facilitation of self-stimulation rates was obtained with racemic ethylketocyclazocine and its (+) enantiomer (Reid *et al.* 1985). On the other hand, reduction of self-stimulation rates was demonstrated with (–) ethylketocyclazocine, which has greater κ activity than the (+) enantiomer (Reid *et al.* 1985).

Other evidence for μ and δ , but not κ , facilitation of BSR has been obtained when selective agonists were applied intracerebrally to rats. Equimolar doses of morphine and (D-Pen)², (D-Pen)⁵ enkephalin (DPDPE; a δ agonist) injected into the ventral tegmental area reduced the reward threshold and shifted the rate frequency function to the left for hypothalamic self-stimulation (Jenck *et al.* 1987a). The κ -selective agonist U50,488 had no significant effect on these BSR parameters, although similar ventral tegmental injections of U50,488 were equieffective with morphine and DPDPE in facilitating stimulation-induced feeding (Jenck *et al.* 1987b). The lack of effect of U50,488 on self-stimulation behaviour suggests that ventral tegmental κ receptors are not involved in the opioid enhancement of BSR. μ and δ involvement in

hypothalamic self-stimulation, however, was additionally supported by the fact that naloxone, which has at least 10-fold greater affinity for μ than δ receptors, was more effective at blocking the μ -based morphine than the δ -based DPDPE effects.

A similar approach has been used to characterize reward-relevant opioid receptor types in the nucleus accumbens (West & Wise 1989). In this instance, the μ -selective agonist (D-Ala¹, MePhe⁴, Gly-ol⁵) enkephalin (DAGO), DPDPE, or U 50,488 were injected into the nucleus accumbens during BSR testing with lateral hypothalamic electrodes. The μ and δ agonists shifted the rate-frequency function to the left with no change in maximum rate, while the κ agonist again had no effect, as was found previously with ventral tegmental injections. On the other hand, De Witte *et al.* (1989) reported that intra-accumbens DAGO significantly reduced hypothalamic self-stimulation, but measurements were made only on a high baseline response rate maintained by a single current intensity.

Curiously, medial thalamic injections of DAGO elevated lateral hypothalamic self-stimulation thresholds, whereas similarly placed injections of U50 488 reduced them (Carr & Bak 1988). It is possible that opioid receptors in the medial thalamus act reciprocally in BSR to those in other brain sites.

Opioid-induced conditioned place preference. Conditioned place preference is a simple conditioning procedure for measuring the reinforcing properties of drugs in animals. Typically, the apparatus is a box consisting of two distinctive chambers. When injections of a reinforcing drug are paired with confinement in one of the chambers over several training sessions, animals apparently learn to associate that place with the drug reward. On subsequent test trials, when the drug is absent, the animals exhibit a preference for the chamber previously associated with the drug. On the other hand, if the drug has aversive properties, the place associated with the drug is avoided. An important advantage of this method is that the animal is tested in the absence of the conditioning drug; hence, direct effects of the drug on test performance may be ruled out. Because of its technical simplicity, the conditioned place preference is widely used for investigating the neuropharmacology of abused drugs.

As in operant paradigms, peripheral applications of μ -preferring agonists such as morphine, fentanyl and sufentanyl exert rewarding actions as measured by a preference for the drug-paired environment (see Hoffman 1989). A place preference has also been demonstrated with intraventricular injections of β -endorphin (Almaric *et al.* 1987), which acts predominantly at μ and δ receptors. Strong evidence for δ -mediated reward was obtained when intraventricular injections of the δ agonist DPDPE and morphine both induced a place preference, and only the DPDPE-induced place preference could be prevented by intraventricular pretreatment with the δ antagonist ICI 174,864 (Shippenberg *et al.* 1987). These results support a role for δ receptors in reward (and again suggest that the reinforcing

effects of μ -preferring morphine are δ -independent). A recent study extended these findings by demonstrating that a place preference could be produced with the μ -selective agonist DAGO; this effect was blocked only by the μ -selective antagonist D-Pen-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂ (CTOP), and not ICI 174,864 (Bals-Kubik *et al.* 1990). Conversely, CTOP pretreatment failed to block a DPDPE place preference, consistent with the inference that μ and δ agonists act at separate recognition sites to produce place preference.

Curiously, either μ or δ antagonists could block the place preference produced by the endogenous peptide β -endorphin, suggesting that joint activation of both μ and δ receptors may be required for β -endorphin place preference (Bals-Kubik *et al.* 1990). In support of this idea, the authors cite results obtained with a β -endorphin (1-27) fragment that could antagonize β -endorphin place preference as well as preferences induced with either μ (DAGO) or δ (DPDPE) agonists (Bals-Kubik *et al.* 1988).

Several place preference studies have been conducted with κ agonists, yielding somewhat ambiguous results. Most of these agonists, including κ -selective U50,488 and U69,593 actually produce place aversion in rats (see Hoffman 1989). In contrast, dose-dependent and naloxone-reversible place preferences have been obtained with κ -preferring ethylketocyclazocine and ketocyclazocine (Iwamoto 1986), while others have found only modest, statistically non-significant increases in place preference with ethylketocyclazocine or trifluadom (Mucha & Herz 1985). A positive place preference also was obtained with intraventricular injections of the endogenous κ -active agonist dynorphin A (1-17) (Iwamoto 1988), but, as already noted, dynorphin A has substantial affinity for all three opioid receptor subtypes. More generally, agonist selectivity for the κ receptor predicts aversion, and this aversion seems to be mediated both peripherally (Bechara & van der Kooy 1987) and centrally (Bals-Kubik *et al.* 1989). Finally, it should be noted that κ -preferring antagonists such as Mr 2266 and 2267 (Bechara & van der Kooy 1987) and 1-WIN 44,441-3 (Iwamoto 1986) can produce a place preference, presumably by blocking the aversive action of endogenous opioids at κ receptors.

In summary, a consistent body of evidence supports a role for central μ and δ , but not κ , receptors in opioid reward. This conclusion is supported by experimental data from three reward paradigms. In general, the most selective ligands have yielded the clearest results. Selective agonists acting at μ and δ receptors supported self-administration behaviour, enhanced the reward value of subthreshold brain stimulation, and clearly induced place preference; conversely, central application of μ or δ -selective antagonists effectively blocked both intracranial opioid self-administration and opioid-induced conditioned place preference. On the other hand, the κ selective agonist U50,488 was not self-administered, and it failed to facilitate BSR or produce a place preference. The moderate reinforcing effects of non-selective κ agonist such as ethylketocyclazocine and ketocyc-

clazocine may well be explained by the partial μ -agonist activity of these agents.

Dopamine receptors.

Two main dopamine receptor subtypes have been identified, D_1 and D_2 , which differ in their pharmacological and biochemical properties (Kebabian & Calne 1979). D_1 receptors activate adenylate cyclase, whereas D_2 inhibit it. Recently, the genes encoding three additional dopamine receptors (D_3 , D_4 , and D_5) have been cloned (Sokoloff *et al.* 1990; Van Tol *et al.* 1991; Sunahara *et al.* 1991). D_3 has strong homology and a similar pharmacological profile to D_1 , and it also stimulates adenylate cyclase. D_3 and D_4 have high homology and similar pharmacological profiles to D_2 , but their transduction systems are unknown. Since D_3 , D_4 , and D_5 are too new for published reports of their role in reward, and because of the overlap in their pharmacological profiles, D_1 is not discriminated from D_3 nor D_2 from D_3 or D_4 in the studies reviewed below.

Stimulant self-administration. Several lines of evidence suggest the involvement of the D_2 receptor in stimulant self-administration. First, pretreatment with D_2 agonists has the same effect as increasing the dose of the self-administered drug, i.e., it produces a reward-indicating increase in the interval between successive self-injections. This was reported both for piribedil pretreatment in amphetamine self-administration (Yokel & Wise 1978) and bromocriptine pretreatment in cocaine self-administration (Hubner & Koob 1990; Kleven & Woolverton 1990). Similarly, the selective D_2 agonist N-0923 dose-dependently increased the interval between successive cocaine self-injections (Self & Stein 1991). Even more explicit evidence of D_2 involvement in stimulant reward is demonstrated by the ability of D_2 -selective agonists to substitute for cocaine or amphetamine in self-administration tests. In the rat, piribedil readily substituted for amphetamine (Yokel & Wise 1978) and bromocriptine readily substituted for cocaine (Wise *et al.* 1990); in the rhesus monkey, piribedil always, and bromocriptine usually, substituted for cocaine and amphetamine (Woolverton *et al.* 1984). Furthermore, drug-naïve rats will learn to self-administer D_2 agonists, such as piribedil (Davis & Smith 1977) or N-0923 (Belluzzi & Stein, unpublished observations). In contrast, the D_1 -selective agonist SKF 38393 invariably failed to substitute for cocaine or amphetamine in rhesus monkey self-administration experiments (Woolverton *et al.* 1984). Indeed, in the rat, SKF 38393 pretreatment increased cocaine self-administration rates and shortened interinfusion intervals, an indication that SKF 38393 may reduce the reinforcing efficacy of cocaine (Self & Stein 1991). These studies with dopamine agonists thus implicate D_2 , but not D_1 , receptors in stimulant self-administration.

On the other hand, studies of the effects of dopamine antagonists on stimulant self-administration implicate both D_1 and D_2 receptors in reinforcement. Low doses of pimozide (D_2 antagonist) prompted compensatory increases in amphetamine or cocaine intake (Yokel & Wise 1975, 1976;

Roberts & Vickers 1984); higher doses disrupted self-administration of apomorphine (Baxter *et al.* 1974), or led to extinction-like responding in rats self-administering amphetamine (Yokel & Wise 1975 & 1976). Similar reward-reducing effects of pimozide were reported in rhesus monkeys self-administering either cocaine or piribedil (Woolverton 1986). In the same study, pretreatment with the D_1 -selective antagonist SCH 23390 caused dose-related decreases (and no evidence of compensatory increases) in cocaine or piribedil self-administration. In contrast to these monkey data, dose-dependent compensatory increases in cocaine self-administration were observed in rats following administration of the D_1 antagonist SCH 23390 (Koob *et al.* 1987). The highly potent D_2 antagonist spiperone also produced compensatory increases, but they reached statistical significance only at one dose level, possibly because higher doses of spiperone produced response suppression. Similarly, when administered directly in the nucleus accumbens, spiperone again caused compensatory increases in cocaine self-administration at low doses and response suppression at high doses (Phillips *et al.* 1983). Roberts & Vickers (1984) also obtained dose-dependent increases in cocaine self-administration following systemic administration of the D_2 -selective antagonists sulpiride and metoclopramide. Thus, both D_1 - and D_2 -selective antagonists can block intravenous self-administration of stimulant drugs, but further work with D_1 antagonists especially is indicated.

Rats will self-administer amphetamine into the nucleus accumbens (Hoebel *et al.* 1983) and cocaine into the medial prefrontal cortex (Goeders *et al.* 1986). The reinforcing effects of intracortical cocaine were blocked by coinfusion with equimolar sulpiride (D_2 antagonist), but not with the D_1 -antagonist SCH 23390 at up to twice equimolar concentrations (Goeders *et al.* 1986). Intracortical self-administration of the D_2 -agonist N-0437 also has been demonstrated (Stein & Belluzzi 1989).

Stimulant-induced conditioned place preference. The place preference studies reveal a pattern of results similar to that found in stimulant self-administration. Thus, place preferences are produced with D_2 -selective agonists such as N-0437 (Gilbert *et al.* 1986), bromocriptine (Beninger *et al.* 1989) and quinpirole (Beninger *et al.* 1989), but not with the D_1 -selective agonist SKF 38393. Indeed, SKF 38393 induced a significant place aversion, which was blocked by either the D_1 antagonist SCH 23390 or the D_2 antagonist metoclopramide (Gilbert *et al.* 1986; Beninger *et al.* 1989). SCH 23390 or metoclopramide also were effective in blocking amphetamine-induced place preference (Leone & De Chiara 1987; Beninger *et al.* 1989). Quinpirole-induced place preference also was attenuated by pretreatment with lower doses of SCH 23390 or metoclopramide, but higher doses of each curiously had no such effect (Beninger *et al.* 1989).

Dopamine receptors in brain-stimulation reward. D_2 involvement in BSR was first suggested by a study (Gallistel & Davis 1983) comparing the effects of nine dopamine antag-

onists on hypothalamic self-stimulation. The effectiveness of the antagonists in blocking reward was highly correlated with their affinity for D_2 , but not D_1 , receptors. Several earlier studies had shown extinction-like response patterns for BSR following high doses of the D_2 antagonists, pimozide (e.g., Liebman & Butcher 1973) or metoclopramide (Fenton & Liebman 1982). Similarly, low doses of pimozide increased BSR thresholds (Zaveries & Setler 1979) or displaced the stimulus-response function to the right without reducing maximal performance (Franklin 1978). Spiperone reduced responding for BSR in several brain areas (Rolls *et al.* 1974). Equivocal findings in BSR experiments with D_2 -selective sulpiride have been reported, but this drug penetrates only poorly into the brain. Systemic administration of raclopride, a lipid soluble derivative of sulpiride with high D_2 selectivity, reduced responding for ventral tegmental BSR at doses that did not interfere with responding for food (Nakajima & Baker 1989). Nucleus accumbens injections of raclopride also were effective against ventral tegmental BSR (Nakajima 1989). Taken together, these data suggest an important role for dopamine D_2 receptors in BSR.

D_1 -receptor involvement in BSR also has been demonstrated. The D_1 -selective antagonist SCH 23390 suppressed responding for stimulation of the medial forebrain bundle, ventral tegmental area, and dorsal raphe nucleus in a dose-dependent manner (Nakajima & McKenzie 1986). Low doses of SCH 23390 caused rightward shifts in the stimulus-response function, suggesting a specific reduction in reinforcement efficacy. In a novel approach to minimize the confounding effects of motor incapacitation, rats were trained to produce hippocampal theta waves to receive hypothalamic stimulation. This non-motoric task also was attenuated by SCH 23390 or pimozide pretreatment (Fantie & Nakajima 1987), again supporting the conclusion that both D_1 and D_2 antagonists can reduce BSR independently of motor impairment. Nakajima (1989) observed intriguing differences in the blockade of operant behaviour produced by the D_1 and D_2 antagonists. The D_2 antagonist raclopride required 10-fold higher doses to block bar-pressing for food than it did to block bar-pressing for brain stimulation; however, changes in the scheduling or density of either the food or brain stimulation reinforcer did not affect raclopride's action. On the other hand, the D_1 antagonist SCH 23390 was equally effective against food reinforcement or BSR, but its blocking action was sensitive to reinforcement density. Based on these results, different mechanisms of D_1 and D_2 regulation were suggested, with D_1 receptors related to the schedule of reinforcement and D_2 receptors related to the type of reinforcement (Nakajima 1989).

Conditioned reinforcement of operant behaviour. The pattern of results with D_1 and D_2 agonists again resembles those obtained in self-administration and place preference. In this test, a neutral stimulus (e.g., tone) is paired with a reinforcing stimulus (food) in several classical conditioning sessions. Later, the conditioned reinforcing properties of the tone are assayed in an operant chamber with two levers,

only one of which delivers the tone when pressed. In untreated animals, the tone provides only weak conditioned reinforcement, as revealed by low lever-pressing rates and only a slight differential response between the two levers. However, after treatment with amphetamine or related stimulants, response rates at the tone-associated lever are selectively enhanced in a dose-dependent manner (Hill 1970). In contrast, the non-selective D_1/D_2 agonist apomorphine increased responding on both levers with no differential effect (Beninger *et al.* 1989). In the same study, amphetamine's robust enhancement of conditioned reinforcement was mimicked by the D_2 agonists bromocriptine and quinpirole, but the D_1 agonist SKF38393 was without effect and failed to increase responding at either lever. No experiments have been carried out on the effects of subtype-selective dopamine antagonists on amphetamine-potentiated conditioned reinforcement, nor has cocaine's effectiveness been assessed in this paradigm.

To summarize the dopamine work, the findings with dopamine agonists implicate D_2 , but not D_1 , receptors in stimulant drug reward, whereas experiments with antagonists implicate both D_1 and D_2 receptors. Thus, D_2 agonists clearly reinforced self-administration behaviour, induced a place preference, and enhanced the conditioned reinforcement of operant behaviour. On the other hand, the D_1 agonist SKF 38393 failed to support self-administration behaviour, produced place aversion rather than place preference, and failed to enhance conditioned reinforcement. (However, although highly selective, SKF 38393 is a partial agonist with only 45% efficacy; Andersen & Jansen 1990). In contrast to these differential actions of agonists, both D_1 and D_2 antagonists reduced the reinforcing efficacy of self-administered stimulant drugs, prevented conditioning of stimulant place preference, and blocked brain stimulation reward.

One way to reconcile these apparently conflicting agonist and antagonist data is to assume — along lines previously proposed for D_1 receptors in motor activation (Waddington & O'Boyle 1989) — that D_1 activity plays an enabling or permissive role in reinforcement. According to this idea, reinforcement processes are directly mediated by D_2 activity, but some minimal level of D_1 "tone" is required for their behavioural expression. In such case, either D_1 or D_2 antagonism would block the reinforcing action of stimulants, but only D_2 agonists (given sufficient endogenous D_1 tone) would mimic them. D_1 enabling effects have been demonstrated electrophysiologically on nucleus accumbens neurons. In these studies, neurones unresponsive to a D_1 agonist alone were capable of responding to a D_2 agonist, but only if sufficient D_1 receptor activation was present (White 1987). Furthermore, because involvement of D_1 receptors has been demonstrated in opioid-induced place preference (Leone & Di Chiara 1987; Shippenberg & Herz 1987), it is possible that D_1 activity also enables the expression of opioid reinforcement processes. If so, reinforcement signals transmitted via a variety of first messenger receptors (dopamine D_2 and μ and δ opioid receptors) might all require some minimal level of D_1 activity for behavioural expression.

Comment

Attempts have been made to identify a common mechanism to explain both stimulant and opioid reinforcement. One idea is that stimulants and opioids act on a common anatomical target—the nucleus accumbens (Koob 1988). A second hypothesis proposes that a final common neurotransmitter, dopamine, mediates the reinforcing actions of stimulant and opioid drugs (Bozarth & Wise 1986; Di Chiara & Imperato 1988). A related idea is that a particular dopamine receptor subtype (D_1) is critical for both opioid and stimulant reward (Leone & Di Chiara 1987; Shippenberg & Herz 1987). Finally, as noted above, a common D_1 enabling or permissive mechanism might be operative in stimulant and opioid reinforcement.

All of these ideas emphasize a convergent mechanism of reinforcement at either the anatomical, neurotransmitter, or receptor levels. Convergence at the second messenger level is furthermore suggested by the specific opioid and dopamine receptor types tentatively identified as reward-relevant in this review. μ and δ opioid and dopamine D_1 receptors belong to a receptor family that inhibits cyclic AMP formation via the guanine nucleotide binding protein, G_i (Limbird 1988). And although D_2 -mediated inhibition of adenylylase activity was not observed in slices of nucleus accumbens (Stoof *et al.* 1987), such inhibition has been demonstrated in homogenates of nucleus accumbens and other reward-related brain regions (Memo *et al.* 1987). In addition to inhibiting adenylylase, the G_i -linked receptor family also inhibits neuronal activity through G protein-activation of K^+ channels (Brown 1990) or inhibition of voltage-sensitive Ca^{2+} channels (Dolphin 1990). It thus is conceivable that dopamine D_2 and μ and δ opioid receptors mediate stimulant and opioid reinforcement, respectively, by activation of the same signal transduction mechanisms. In this regard, it is interesting that chronic regimens of morphine or cocaine produced similar changes in nucleus accumbens levels of G_i (but not G_o), adenylylase, cyclic AMP-dependent protein kinase, and a number of cyclic AMP-regulated phosphoproteins (Terwilliger *et al.* 1991).

In summary, μ and δ receptors appear to mediate the reinforcing effects of opioids. The dopamine receptor subtype responsible for stimulant reward is less clear. The data favour D_2 mediation of reinforcement with a permissive or modulatory role for D_1 receptors. All of these receptor subtypes are linked to cyclic AMP formation. Interestingly, α_1 adrenergic receptors, another member of the family of G-linked receptors, also may be involved in reinforcement functions, as suggested by reports that the α_2 -agonist clonidine is self-administered (Davis & Smith 1977; Woolverton *et al.* 1982). Similarly, the positively reinforcing compounds in marijuana, typified by Δ^9 -tetrahydrocannabinol, activate a recently characterized cannabinoid receptor that inhibits adenylylase via G_i proteins (Howlett *et al.* 1986; Matsuda *et al.* 1990). Finally, the 5-hydroxytryptamine $5HT_{1A}$ -selective agonist 8-OH-DPAT has been shown recently to induce a positive place preference (Shippenberg 1991). The

$5HT_{1A}$ receptor also inhibits adenylylase via G_i proteins (Schmidt & Peroutka 1989).

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BRES 25213

The D₁ agonists SKF 82958 and SKF 77434 are self-administered by rats

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The reported failure of the prototypical (but partial) D₁ agonist SKF 38393 to support self-administration behavior contradicts hypotheses of D₁-mediated reinforcement. Here we demonstrate that rats will readily self-administer two SKF 38393 analogs, the partial D₁ agonist SKF 77434 and the full D₁ agonist SKF 82958; both compounds produce inverted U-shaped dose-response curves. When compared to the parent compound, both analogs display enhanced lipophilicity and somewhat decreased D₁/D₂ selectivity. It is suggested that these properties, rather than partial D₁ agonist efficacy, explain the failure of SKF 38393 to act as a reinforcer.

Stimulant drugs of abuse, such as amphetamine and cocaine, are thought to exert their reinforcing actions principally through enhancement of mesolimbic dopaminergic transmission¹⁸. Five dopamine receptor subtypes have been identified to date, which may be classified on the basis of structural homology, biochemical properties and pharmacological profiles into two subgroups ('D₁-like' and 'D₂-like'). A number of agonists and antagonists have been developed that act selectively at either the D₁-like or D₂-like receptors. These ligands have been useful in the investigation of the roles of the D₁ and D₂ subgroups in behavioral reinforcement (for review, see ref. 16).

In general, the findings with dopamine antagonists implicate both D₁ and D₂ receptors in reinforcement. Thus, both D₁ and D₂ antagonists reduced the reinforcing efficacy of self-administered stimulant drugs^{7,15,17,19}, prevented conditioning of stimulant place preference^{2,8}, and blocked brain stimulation reward^{4,10}. On the other hand, experiments with agonists tend to implicate D₂, but not D₁, receptors in reinforcement. Thus several D₂ agonists were reported to be self-administered²⁰, to induce a place preference², and to enhance the conditioned reinforcement of operant behavior². In contrast, the single D₁ agonist (SKF 38393) tested to date for its reinforcing properties has failed to support self-administration behavior²⁰, has produced place aversion rather than place preference^{2,5}, and has failed to enhance conditioned reinforcement². In view of the reinforcement-blocking actions of D₁ antagonists, the failure of SKF 38393 to act as a positive reinforcer is puzzling and it constitutes the

most important negative evidence against the hypothesis that D₁ receptor activation mediates reinforcement.

Although commonly regarded as the prototypical D₁ agonist, SKF 38393 is a partial agonist with only 45% efficacy in stimulating D₁-sensitive adenylate cyclase^{1,11}; furthermore, SKF 38393 may have only a limited ability to penetrate the blood brain barrier¹². A better test of D₁ involvement in behavioral reinforcement would be provided by SKF 82958, which not only is a full D₁ agonist^{11,12}, but which also has greater ability to penetrate into the brain than SKF 38393¹². In addition, SKF 82958 produces the grooming behavior characteristic of a D₁ agonist⁹. In the present paper, we demonstrate that SKF 82958 in fact is a powerful reinforcer that is avidly self-administered by rats.

Subjects were experimentally naive, individually housed, male, Sprague-Dawley rats (270–300 g) who were initially trained to press a lever for food pellets. After 3 days of lever-press training, the rats were surgically implanted with a chronically indwelling jugular catheter prepared from Silastic tubing. Following a 4-day recovery period, animals were placed in an operant test chamber and connected to a syringe pump system¹⁴. A 10 g lever-press response delivered a 0.1 ml intravenous injection of sterile-filtered drug solution. A clearly audible tone was sounded during the 6 s injection interval. Each self-injection response was followed by a 10 s 'time out' period in which the box light was extinguished and lever-press responses had no programmed consequences. The test procedures were automatically controlled by an IBM PC 30 computer and Lab Line I/O panel (Coul-

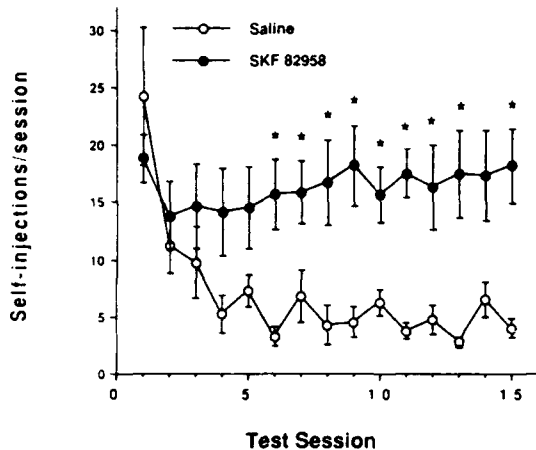


Fig. 1. Self-administration of SKF 82958 in drug-naïve rats. Data points show the mean daily number of self-injections (\pm S.E.M.) of groups self-administering SKF 82958 at a dose of 10 μ g/kg/injection ($n = 6$) or saline ($n = 4$) over 15 consecutive test sessions (* SKF 82958 differs from saline, $P < 0.05$, Student's t -test). High response rates in the initial test sessions are due to the fact that all rats were trained to lever-press for food pellets prior to self-administration testing.

naïve animals would self-administer SKF 82958 (6-chloro-*N*-allyl-SKF-38393 HBr; Research Biochemicals Inc., Natick, MA). Different groups of animals, which previously had received a food pellet after each lever-press response, now received instead an intravenous injection either of SKF 82958 (10 μ g/kg) or of the saline vehicle. A total of 15 daily 3 h test sessions were given. As shown in Fig. 1, the group receiving SKF 82958 exhibited sustained self-administration behavior throughout the 15 test sessions, while the response rate of the saline controls declined rapidly (presumably, as a result of extinction) from the high levels maintained by prior food reinforcement. Two-way ANOVA with repeated measures (test session) revealed a significant main effect for drug treatment (SKF 82958 vs. saline; $F_{1,8} = 7.740$, $P = 0.024$). A significant treatment \times test session interaction also was obtained, reflecting the differential course of responding over test sessions in the two groups ($F_{14,112} = 3.147$, $P < 0.001$). Inspection of individual rat data revealed that 5 of the 6 SKF 82958 rats maintained stable self-administration rates throughout the experiment.

In a second experiment, various doses of SKF 82958 were tested for self-administration using 4 of the animals from experiment 1 that had exhibited stable SKF 82958 self-administration, and 2 new SKF 82958 self-administrators that had been trained initially to self-administer cocaine. Each dose of SKF 82958 (or saline) was offered in two consecutive, 3 h self-administration tests employing different sequences for each animal: to minimize transition effects, only data from the second test was used for analysis. Finally, 4 of the rats were tested with

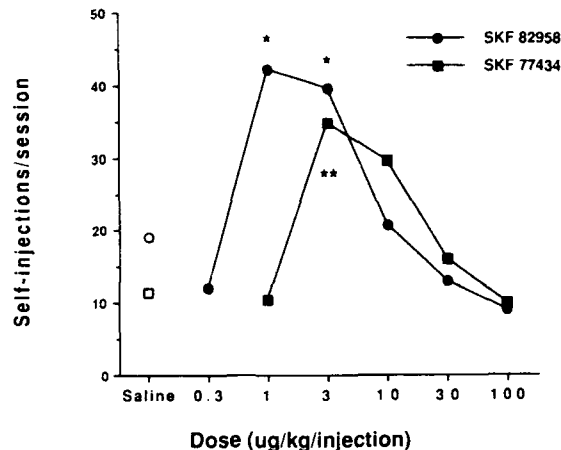


Fig. 2. Dose-response curves of SKF 82958 and SKF 77434 self-administration. Data points show the mean number of self-injections of groups offered various doses of SKF 82958 ($n = 6$) or SKF 77434 ($n = 4$). Open symbols indicate saline means of respective groups. To minimize carry-over effects, only data from the second of two consecutive 3 h tests of each dose are plotted (* SKF 82958 differs from saline, $P < 0.01$; ** SKF 77434 differs from saline, $P < 0.05$, Newman-Keuls' test).

various doses of SKF 77434 (*N*-allyl-SKF 38393 HCl; Research Biochemicals Inc., Natick, MA), an analog that has similar lipophilicity to SKF 82958 and similar partial D_1 agonist efficacy to SKF 38393.

Inverted U-shaped self-administration dose-response curves were obtained for both SKF 82958 and SKF 77434 (Fig. 2). SKF 82958 was both more potent and more efficacious than SKF 77434, as indicated by the leftward displacement and higher peak rate of the SKF 82958 self-administration curve. One-way ANOVA with repeated measures revealed significant effects of dose for SKF 82958 ($F_{6,30} = 10.965$, $P < 0.001$) and SKF 77434 ($F_{6,18} = 5.547$, $P = 0.002$). The inverted U-shaped dose-response curves obtained with SKF 82958 and SKF 77434 resemble those seen with many other self-administered

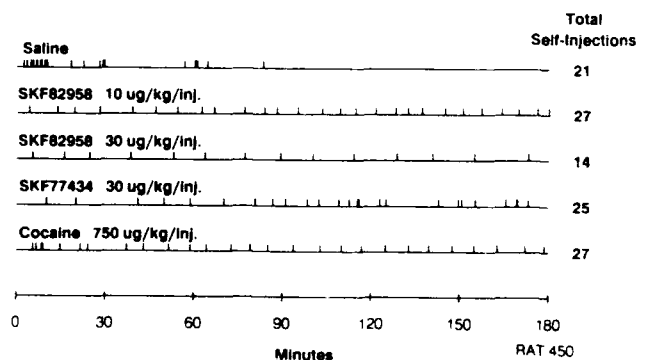


Fig. 3. Event records of a representative rat during 3 h self-administration tests with saline, SKF 82958, SKF 77434, or cocaine as reinforcers. Deflections mark the times of each self-injection response.

response curves obtained with SKF 82958 and SKF 77434 resemble those seen with many other self-administered compounds¹³, and reflect the fact that an intermediate dose level produces a maximum rate of self-administration. Rates are reduced at higher doses, presumably because the effects of each injection are prolonged. Self-administration rates also are reduced at lower doses, presumably because the reinforcing efficacy of each injection is lessened; indeed, the lowest dose of each compound produced the same low rates and irregular response patterns as those produced by saline.

SKF 82958 self-administration was characterized by relatively regular interinfusion intervals, a pattern which also is typical of cocaine (Fig. 3). One notable difference is that in the case of cocaine, the beginning of each self-administration session is usually marked by a brief period of rapid response (which, it is speculated, brings blood cocaine levels quickly to preferred levels³). In contrast, such initial rapid responding was not observed with the D₁ agonists (Fig. 3); indeed, in every rat tested, the rate of self-administration actually increased throughout the test session. Thus, for example, at the standard 10 µg/kg/injection dose of SKF 82958, a mean of 5.5 ± 1.06 self-injections was obtained in the first hour of testing, 6.67 ± 1.28 in the second hour, and 8.67 ± 1.18 in the third hour. One-way ANOVA with repeated measures revealed that these within-session increases in self-administration were statistically significant ($F_{2,10} = 16.687$, $P < 0.001$).

The present results suggest that the partial efficacy of the D₁ agonist SKF 38393 is not the decisive factor in its lack of reinforcing efficacy. SKF 77434 has similar partial D₁ agonist efficacy as SKF 38393^{1,11}, yet we find that it is readily self-administered. (However, it should be

noted that SKF 77434 was self-administered with lower potency and somewhat less reliably than the full agonist SKF 82958.) It may also be noted that SKF 82958 and SKF 77434 both contain an *N*-allyl substitution that increases the lipophilicity of these compounds relative to that of SKF 38393¹²; hence, the substituted compounds penetrate the blood-brain barrier more rapidly than the parent compound. Rapid penetration into the brain (as produced, for example, by a favorable route of administration) is known to be a determining factor in a drug's reinforcing efficacy⁶. Hence, it is reasonable to assume that the ability of SKF 82958 and SKF 77434 to support self-administration behavior is associated at least in part with their high lipophilicity. If so, the inability of the prototypical D₁ agonist SKF 38393 to support self-administration would be due to its low lipophilicity.

This conclusion must be tempered somewhat in view of the fact that, in the rat, the allyl-substituted compounds exhibit less D₁/D₂ selectivity than SKF 38393. Thus, in rat striatal membranes, SKF 82958 and SKF 77434 are reported to be from 3- to 25-fold less selective for D₁ over D₂ receptors than SKF 38393^{1,9}. This somewhat reduced selectivity may explain the fact that SKF 82958 and SKF 77434 stimulate locomotor (D₂-related?) activity at doses that induce intense grooming (D₁-related?) responses⁹. Thus, the reinforcing properties of SKF 82958 and SKF 77434 may not be attributed exclusively to their D₁ receptor activity, since the possibility of D₂ activation cannot be entirely ruled out.

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DRAFT

OPERANT-CONDITIONING OF HIPPOCAMPAL CA1 NEURONS WITH N-0923, A
DOPAMINE D₂ RECEPTOR AGONIST, AS REINFORCEMENT

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ABSTRACT

Previous work indicates that burst activity of individual hippocampal CA1 cells can be reinforced by local micropressure injection of dopamine. The work reported here evaluated the reinforcing effects of N-0923, a selective dopamine D₂ receptor agonist, in cellular operant conditioning. Using the hippocampal-slice preparation, we recorded extracellularly the spontaneous bursting of individual CA1 pyramidal neurons with micropipettes containing the drug solution. Activity-contingent administration of drugs using local micropressure injections was compared with identical injections administered in the absence of burst activity. Reinforcement (increased incidence of bursts) was demonstrated with activity-contingent application of N-0923 (3- and 6-mM pipette concentration) or dopamine (1 mM), but not with the inactive isomer of N-0923 (N-0924; 6 mM). Non-contingent applications of N-0923 or dopamine had no reinforcing action. The reinforcing action of N-0923 on hippocampal CA1 cells was blocked by sulpiride (10 mM), but not by SCH 23390 (1 mM). These results are consistent with the idea that dopamine may play an important role in operant conditioning of hippocampal CA1 neurons and that dopamine's reinforcing action on neuronal bursting may be exerted at dopamine D₂ receptor.

<Key Words: Reinforcement, Dopamine, Hippocampal CA1 cell, D₂ Receptor Agonist, operant conditioning>

INTRODUCTION

The presence of dopamine in the hippocampus was demonstrated relatively late in comparison with other brain areas (1, 9). Moreover, its function in this brain structure was arguable mainly due to a low physiological concentration (11). Electrophysiological studies have provided direct evidence of either a transmitting or neuromodulating role of dopamine in the hippocampus (16, 23). It has been found that dopamine affects the membrane potential, as well as evoking activities of CA1 pyramids (16, 17, 30). One study showed that the intrahippocampal injections of dopamine receptor agonist evoked the theta rhythm in the hippocampal electroencephalogram (24). This effect can be correlated with the behavioral arousal observed under similar experimental conditions (25). Besides, hippocampus has been used to investigate cellular mechanism that may underlie changes in synaptic efficacy as well as learning and memory (7, 32). Hippocampal pyramidal neurons show pronounced long term potentiation (32) and their regulation by biogenic amines (8, 10, 18, 22) as well as the evidence that dopamine agonists could function as a positive reinforcer in animals (2, 15, 34, 37) has led us to study the reinforcing effects of dopamine on hippocampal neurons. However, there is little evidence (3, 4, 27) available for the operant conditioning in hippocampal individual neurons resulting from the limitation of technique (13, 36). Our previous studies (27) first revealed that the spontaneous bursting of individual CA1 pyramidal neurons was increased with activity contingent injections of dopamine. Since it was shown subsequently that reinforcement of dopamine on hippocampal CA1 neurons is dopamine D₂ receptor specific, the present study was

undertaken to evaluate the reinforcing effects of N-0923 (a selective D₂ receptor agonist) and to compare its effects with dopamine on the operant conditioning of CA1 neurons in hippocampal slices.

METHODS AND MATERIAL

The experiments were performed on transverse hippocampal slices prepared from male Sprague-Dawley rats (200-270 g). The rats were lightly anesthetized with Halothane and decapitated. The brain was removed rapidly from the skull and allowed to cool at 4 °C in artificial cerebrospinal fluid (ACSF) containing NaCl (124 mM), KCl (5 mM), CaCl₂ (2.4 mM), MgSO₄ (2 mM), KH₂PO₄ (1.25 mM), NaHCO₃ (26 mM) and glucose (10 mM). The hippocampus was dissected out and sliced into 400-μM slices using a McIlwain tissue chopper. Using an eyedropper, 6-8 slices were individually transferred to a static chamber where they were supported on nylon mesh at the surface of the ACSF solution in an oxygenated atmosphere (95% O₂, 5% CO₂) at 35 °C. The ACSF solution in the static chamber was changed every 30 min, unless prohibited by potential disruption of an ongoing experiment. Following incubation for at least 2 hr, cellular activity was recorded using single-barrelled extracellular micropipettes filled with vehicle or drug solution and with the tip broken to permit pressure ejection of a 10 μ-diameter droplet following a 50-msec application of nitrogen at 15 P.S.I. During operant conditioning, micropressure injections of drug were applied directly to the cell for 50 msec following bursts of activity. Drug-induced increases in bursting are necessary but not sufficient evidence of cellular operant conditioning, since the drug treatments may directly stimulate or facilitate cellular firing. As a mandatory control for such pharmacological stimulation, the same drug injections must be administered independently of bursting on a noncontingent or random basis. Cellular reinforcing effects may be inferred only if the noncontingent injections are relatively

ineffective. The experimental setup is shown diagrammatically in Fig 1. A burst was defined as a train of firing containing N or more spikes with a maximum interspike interval (ISI) of t msec. Normally, reinforceable bursts of activity contained 3-6 spikes with a maximum ISI of 10 msec. The parameters were set individually for each test neuron such that bursts occurred at a baseline rate of approximately 5 per min.

A complete neuronal operant conditioning experiment involved six stages: *Baseline*: the rate of bursting prior to operant conditioning was determined in a baseline period of approximately 5-10 minutes. *Reinforcement*: each burst was now followed by an injection of the test solution. To minimize injection artifacts, neuronal activity during and for 3 sec after each injection was excluded from analysis and had no programmed consequences. *Extinction*: reinforcement was terminated and recording continued until the baseline burst rate was recovered. *Matched (Free) Injections*: noncontingent injections of the test solution were given at regular intervals to determine the direct pharmacological effects on neuronal activity. The number of injections was matched to the 3-5 highest injection rates received during the prior reinforcement period. Again, neuronal activity during and for 3 sec after each injection was excluded from analysis. *Washout*: a second baseline period was given in order to allow residual effects of drug administration to dissipate and for baseline burst rates to return. *Reacquisition*: a second period of reinforcement was scheduled, whenever possible, in order to compare rates of original acquisition and reacquisition and to ascertain the viability of the preparation following noncontingent injections.

A stock solution of CP-55,940 at 10 mM in absolute ethanol was stored at -20 °C. Test solutions were prepared by diluting the stock solution with saline to final CP-55,940

concentrations of 2.5, 5, 10 and 100 μ M. Vehicle-control tests were performed with saline containing the same concentration of ethanol (0.1%) as the optimally-reinforcing 10 μ M CP-55,940 solution.

RESULTS

Fig. 2 shows the results from a typical reinforcement experiment on rat hippocampal slice CA1 neurons. It can be seen that in two separate periods of operant conditioning (REINF), the frequency of bursts and the overall firing rate were rapidly increased after several contingent applications of dopamine (1 mM pipette concentration) reinforcement. The same dopamine dose administered noncontingently (MATCH) failed to increase either frequency or overall firing rate. More than 60% of the tested neurons were successfully reinforced by burst-contingent application of dopamine. This result is consistent with our previous findings (3, 28). In a second experiment, a highly specific dopamine D₂ agonist, N-0923, was compared for ability to reinforce CA1 cells bursting. Fig. 3 shows a typical experiment in which the frequency of bursts and the overall firing rate in hippocampal CA1 cells was increased rapidly and significantly following several reinforcements with N-0923 (6 mM). The N-0923 injections administered noncontingently (FREE) did not increase either the burst frequency or the overall firing rate. N-0923 reinforced CA1 cell bursting and the overall firing in a dose-dependent manner. Three doses of N-0923 (1, 3 and 6 mM) were tested and compared with the optimal dopamine dose (1 mM); weak activity was obtained at the lowest dose of N-0923, but robust reinforcement was demonstrated with 3 and 6 mM N-0923 (Fig. 4). N-0924 (the inactive stereoisomer of N-0923) in a dose of 6 mM had no effects on the operant conditioning in hippocampal CA1 cells. When we compared reinforcing effects of N-0923 with dopamine on hippocampal slice CA1 cells, these data (Fig. 4) indicated that the ability of N-0923 is the same as dopamine in terms of operant

conditioning on hippocampal CA1 bursting.

To determine further whether the specificity of N-0923 for D₂ receptor in the hippocampal CA1 cells and examine whether dopamine's reinforcing action is specifically exerted at the D₂ receptor, dopamine antagonists were studied in the cellular operant conditioning experiment. The selective D₁ dopamine receptor antagonist SCH 23390 was mixed with N-0923 (N-0923 + SCH), the reinforcing action of N-0923 was unaffected, but when the selective D₂ receptor antagonist sulpiride was added to N-0923 (N-0923 + SUL) the reinforcing action of N-0923 was abolished (Fig. 5). These results are consistent with the our pervious findings (3, 28) that dopamine's reinforcing action on hippocampal bursting can be attributed to specific stimulation and is exerted at dopamine D₂ receptors. Moreover, these results indicate that N-0923 may be substituted for dopamine as an effective reinforcer in neuronal operant conditioning.

DISCUSSION

Previous studies suggested that dopamine receptors are two types, D_1 and D_2 . Dopamine D_2 receptors are of primary importance in the reinforcing effects of psychomotor stimulants (34, 35) and the experiments with D_2 antagonists have been consistent with this conclusion (19, 36). However, little is known about the reinforcing action of dopamine on the hippocampal CA1 single unit. We had demonstrated earlier that the spontaneous bursting of CA1 pyramidal neurons may be reinforced with contingent injections of dopamine. The reinforcing action of dopamine is specific and exerted at D_2 receptors (27, 3, 4). The experiments presented above show that a highly specific D_2 agonist, N-0923 can successfully reinforce the hippocampal slice CA1 cell contingent bursting. N-0924, the optical isomer of N-0923 with 100-fold less potency as a D_2 agonist in a behavioral stereotypy assay, was inactive at 6 mM. The reinforcing action of N-0923 suggests that reinforcement of hippocampal CA1 cellular activity was exerted at D_2 receptors since N-0923 is 15-fold selective for D_2 receptors. Our present results obtained with the selective D_2 receptor antagonist sulpiride, as well as D_1 antagonist SCH23390, support this assumption. However, there is a major concern that dopamine and N-0923 may act merely by direct or indirect pharmacological stimulation or facilitation of bursting, rather than by some activity dependent process analogous to behavioral operant conditioning. Because hippocampal CA1 cells bursting was not increased by noncontingent administration dopamine and its agonists, we can rule out the possibility that direct stimulant effects of dopamine caused the increase in neuronal activity that were observed in the reinforcement periods. On the other

hand, most electrophysiological studies showed that the activity of CA1 cells was decreased by local micropressure applications of dopamine (5, 6, 26). Dopamine produced a suppression of hippocampal CA1 cells accompanied by membrane hyperpolarization. Some authors demonstrated (16) a biphasic effect of dopamine on the membrane potential and population spikes in hippocampal slice CA1 cells. The hyperpolarization accompanying inhibition of both the spontaneous and evoked activity was seen immediately following application of dopamine followed a late developing depolarizing induced by dopamine occurring one hour later. The reinforcement experiment we observed with dopamine or N-0923 was within 30-60 minutes. Our results indicate that the reinforcing effect of dopamine on hippocampal slice CA1 bursting is exerted at D₂ receptors. D₂ receptors were originally thought not to be linked to adenylate cyclase, but recently they have been found to be negatively linked to adenylate cyclase and D₂ agonists and can inhibit the production of cAMP (29). More recently, some studies have shown that dopamine D₂ receptors involve both cellular and behavioral reinforcement activity via pertussis toxin-sensitive G-protein or regulatory protein to inhibit adenylate cyclase activity and to enhance potassium conductance (12, 14, 20, 21, 30). It is possible that the intracellular changes associated with dopamine reinforcement involve a decrease in cAMP availability, or perhaps the involvement of cyclic AMP in the reinforcement action of the CA1 pyramidal cell induced by dopamine receptors requires further study. In conclusion, the N-0923 (D₂ agonist) acts as a potent specific reinforcer of dopamine's action on hippocampal CA1 neurons, may be a useful drug for *in vivo* studies designed to assess the pharmacological and reinforcement action of dopamine on behavioral study. The reinforcement action of N-0923 on

hippocampal slice CA1 pyramidal neurons is stereospecific and exerted at D₂ receptors.

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FIGURE CAPTIONS

FIGURE 1. Schematic diagram of cellular operant-conditioning experiment. A single-barrelled glass micropipette for simultaneous recording and pressure injection is filled with dopamine (1 mM in 165 mM saline) or other drugs and aimed at spontaneously active hippocampal cells in the CA1 layer. Amplified action potentials are processed by a spike enhancer and window discriminator (not shown) to increase the signal-to-noise ratio and to isolate signals when multiple-unit activity is encountered. When the computer recognizes a reinforceable burst of activity (based on criteria established individually for each test neuron before operant conditioning), the pressure-injection pump is activated for 5-50 msec to deliver an approximately 10 μ -diameter droplet of drug in the close vicinity of the cell. Drug-induced increases in bursting are necessary but not sufficient evidence of cellular operant conditioning, since the chemical treatments may directly stimulate or facilitate cellular firing. As a mandatory control for such pharmacological stimulation, the same drug injections are also administered independently of bursting on a noncontingent or "free" basis. Cellular-reinforcing effects may be inferred only if the noncontingent injections are relatively ineffective (28). (Upper trace) Burst of firing recorded extracellularly from a CA1 cell exhibiting typical decreasing pattern with progressively shorter and broader spikes occurring later in the burst. (Lower trace) 1-msec logic pulses triggered by each spike. Spikes that fall in the discriminator window are converted to logic pulses for counting by the computer.

FIGURE 2. Operant conditioning of the activity of a CA1 pyramidal cell in a slice of dorsal

hippocampus with local injections of dopamine used as reinforcement. The activity of the unit throughout seven phases of a complete experiment is shown. Each point shows the number of bursts (lower graph, ■) or the total number of spikes (upper graph, •) in successive blocks of 100 half-second samples or trials. Prior to the first baseline phase, a burst criterion of 4 or more spikes per half-second sample was selected. This criterion gave a burst rate for this unit that never exceeded 8% in the initial baseline period (BASE). In the reinforcement period (REINF), dopamine HCl (1 mM in 165 mM saline) was applied for 5 msec immediately after each burst and the burst rate increased to a maximum of 42%. Following a second baseline period, the same number of dopamine injections were delivered (MATCH) independently of the cell's burst activity as a control for possible direct stimulant effects of dopamine injections. The number of injections was matched to that earned during the last four periods of the reinforcement phase. Rates of bursting and overall firing were increased by the contingent dopamine injections during the reinforcement periods, but were not increased when the same injections were administered noncontingently in the matched-injection period.

FIGURE 3. Operant conditioning of a CA1 pyramidal neuron in a dorsal hippocampal slice using local injections of N-0923 as reinforcement. For details, see FIG.2

FIGURE 4. Activity-contingent application of N-0923 produces a dose-dependent increase in burst activity (REINFORCEMENT). Activity-contingent application of the same dose of N-0924, the inactive optical isomer, or of the saline vehicle (0) has no effect on burst

rate. Non-contingent application of N-0923 (FREE) has a significant inhibitory effect on burst rate at the low dose and no effect at higher doses. Mean No. of bursts is calculated by averaging the two highest 100-trial (or 50-sec) burst scores recorded for each unit and then averaging these values for each treatment group. ** Differs from saline-reinf, $p < 0.01$; * Differs from saline-reinf, $p < 0.05$.

FIGURE 5. The selective dopamine D_2 receptor antagonist sulpiride, but not the selective D_1 antagonist SCH23390, blocks CA1 cellular operant conditioning in hippocampal slices with local injections both of the dopamine and N-0923 as reinforcement. Neurons reinforced with 1 mM dopamine (DA) or 6 mM N-0923, exhibited significantly ($p <$) more bursts than controls reinforced with saline (SALINE). When sulpiride (10 mM) was added to the N-0923 solution (N + SUL), the reinforcing action of N-0923 was abolished. On the other hand, when 1 mM SCH23390 was added to the N-0923 solution (N + SCH), the reinforcing action of N-0923 was significantly ($p <$) greater than saline. Bars show peak rates of bursting, which are calculated by averaging the two highest 100-trial (or 50-sec) bursting scores recorded for each unit, and then averaging these values for each treatment group. ** Differs from saline-reinf, $p < 0.01$; * Differs from saline-reinf, $p < 0.05$.

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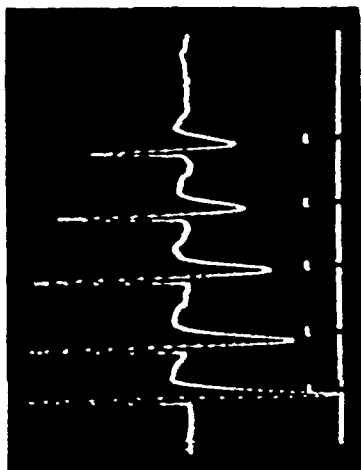
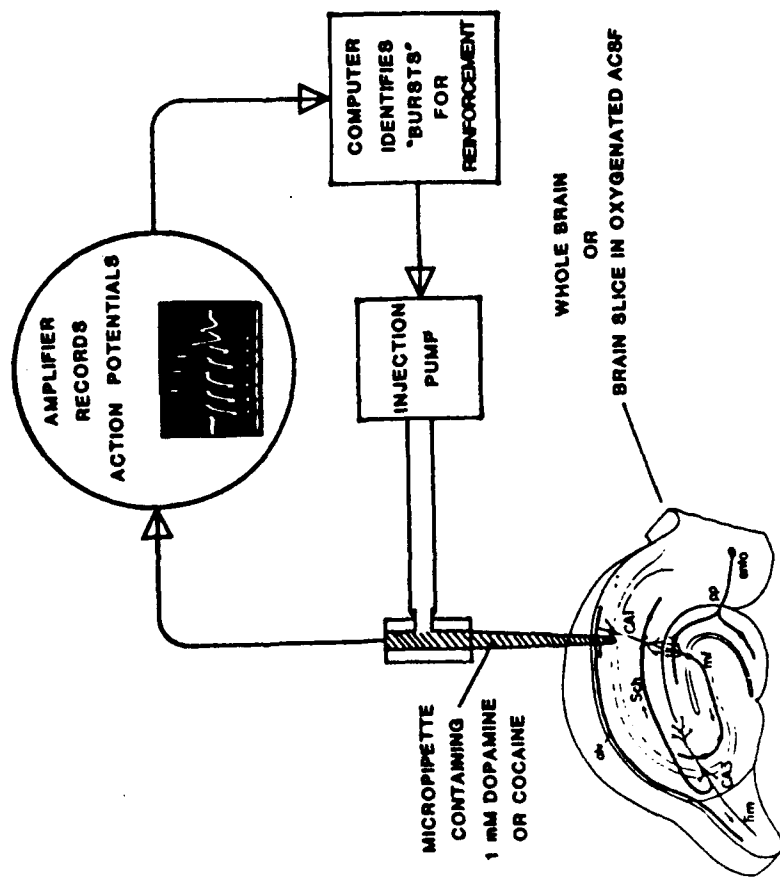
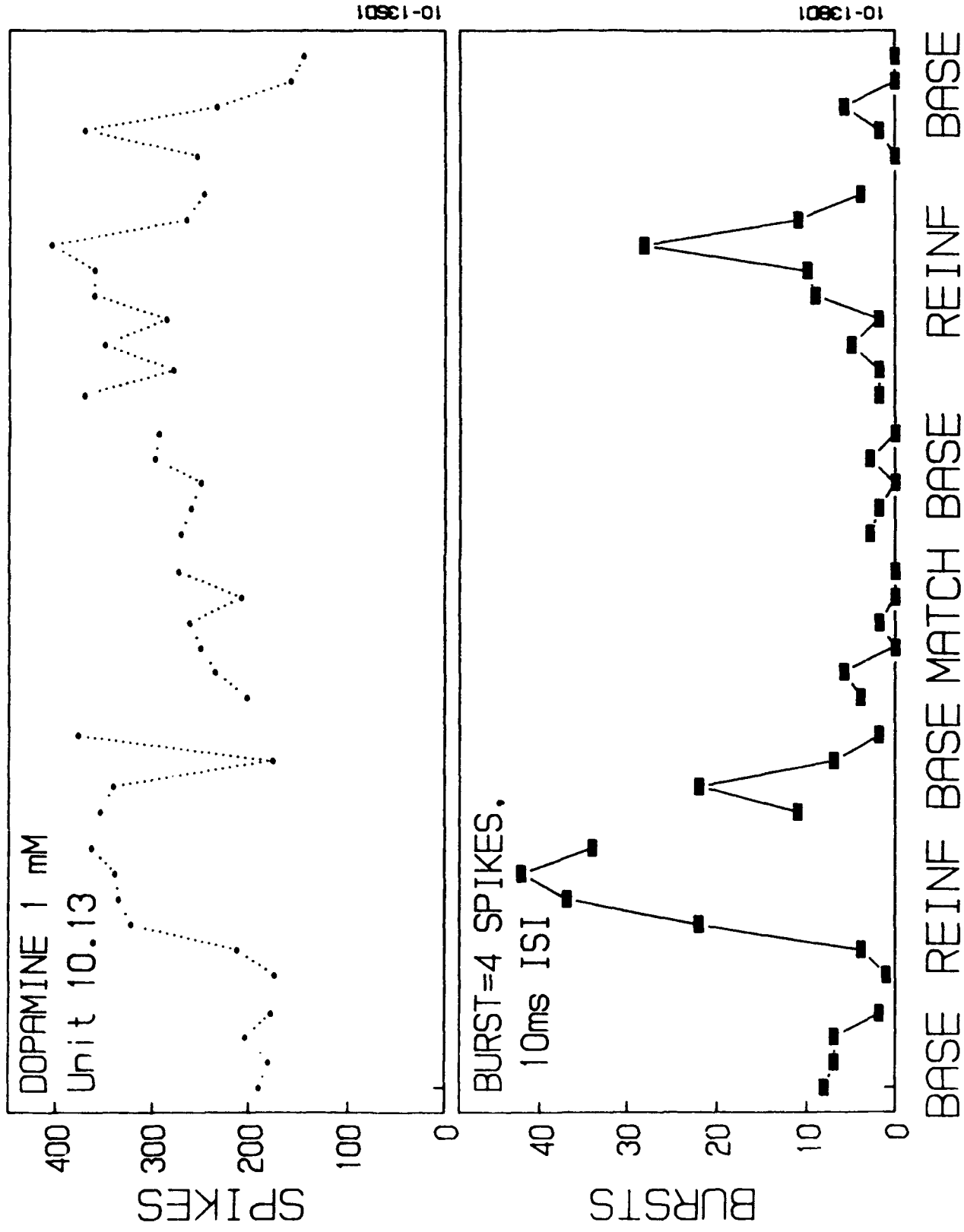
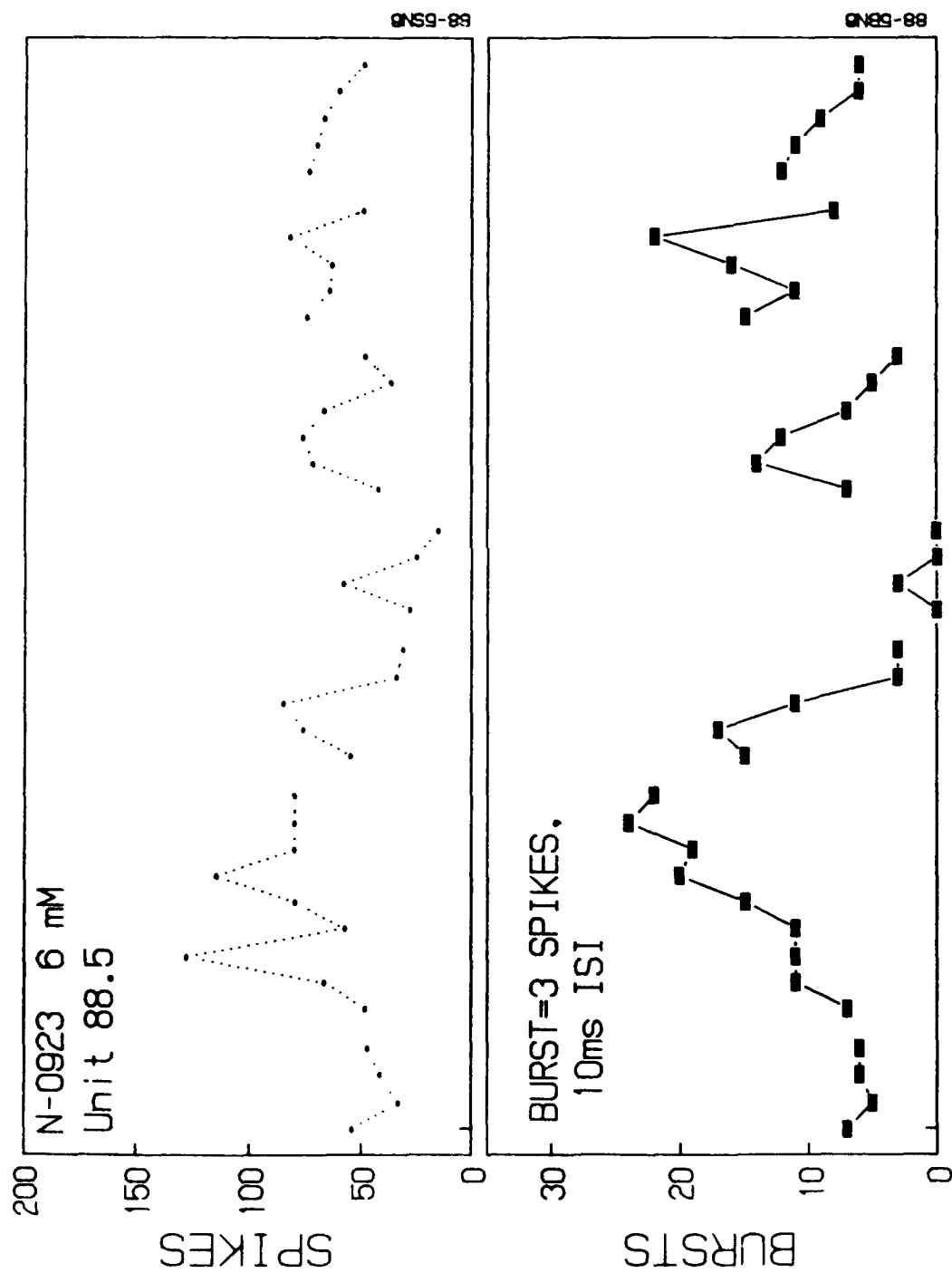
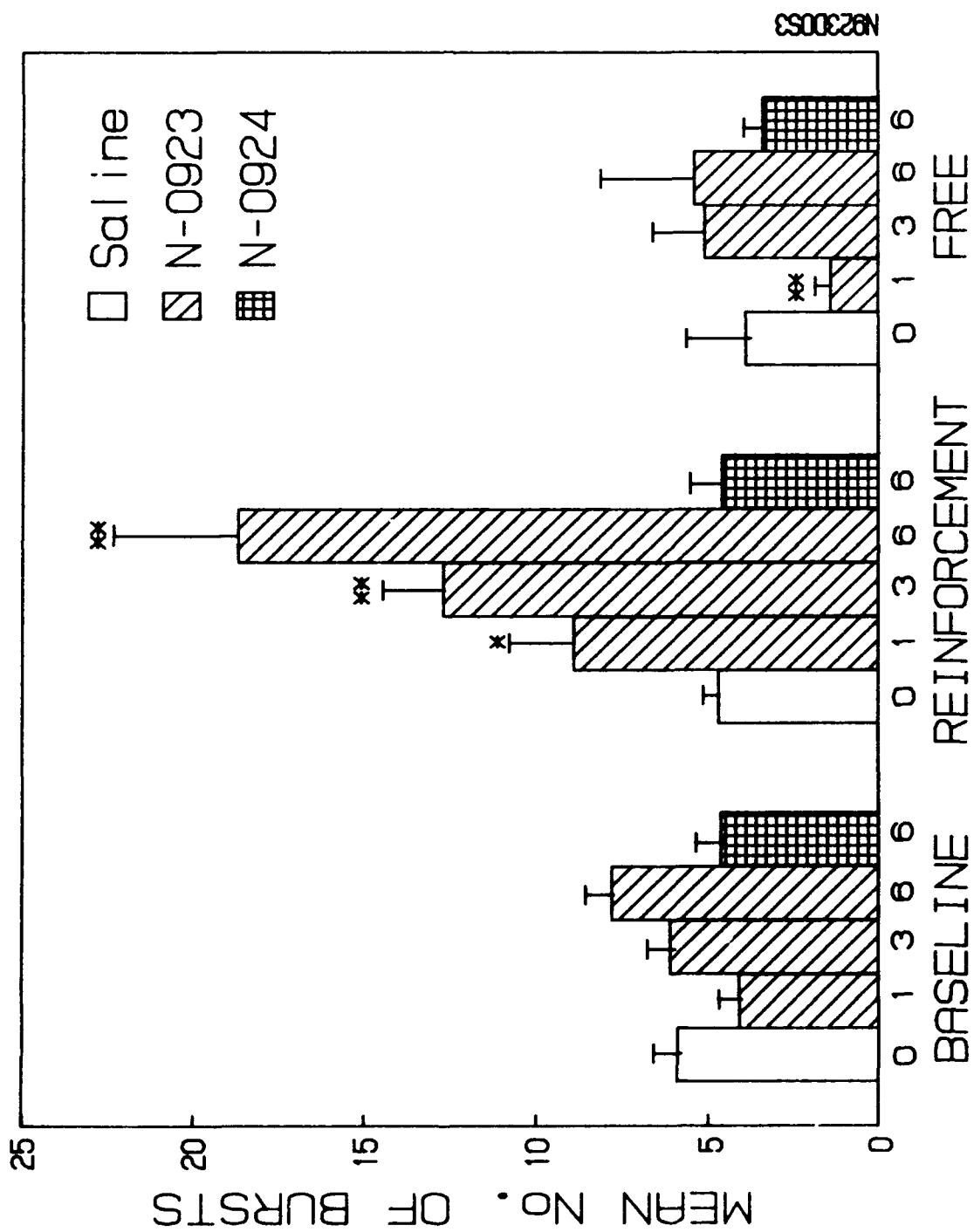


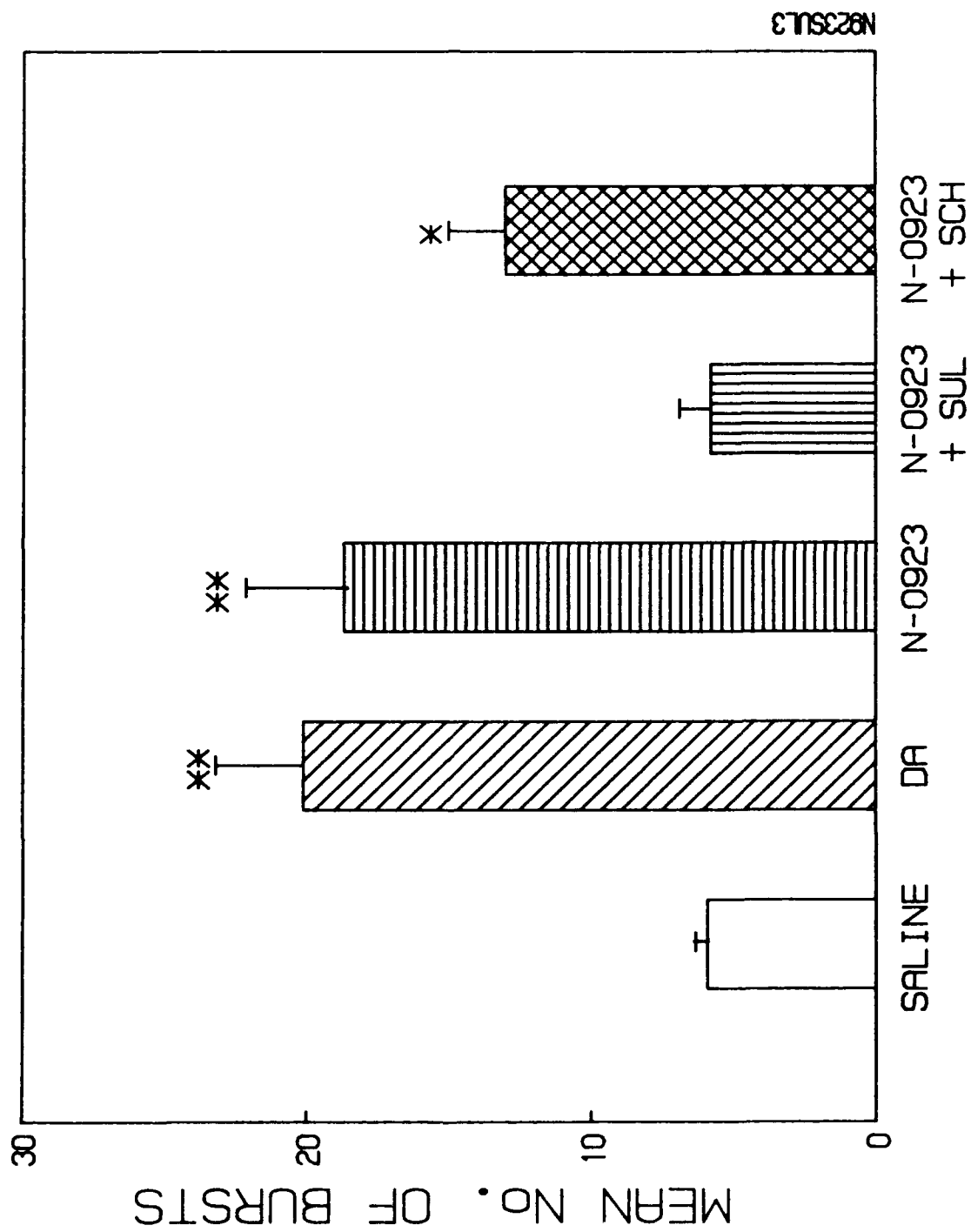
Fig 1





BASE REINF BASE MATCH BASE REINF BASE
SUCCESSIVE 1-MIN SAMPLES





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Hippocampal μ -receptors mediate opioid reinforcement in the CA3 region

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Key words: Self-administration; Hippocampus; Opiate; Dynorphin A; μ -Receptor; ICI 174,864; Nor-binaltorphimine; β -Funaltrexamine

Dependence on reinforcing chemicals is manifested when drug-seeking and drug-taking behaviors come to dominate the response repertoire. Clinical observations suggest that the craving and compulsive drug-seeking that characterize drug dependence are aroused by memories of the reinforcing drug experience. If so, a brain structure intimately associated with memory — the hippocampus — would be a plausible substrate for drug reinforcement effects. We report here that drug-naïve rats rapidly learn to self-administer the opioid peptide dynorphin A in the CA3 region of hippocampus, and that this behavior is blocked by co-administration of the non-selective opiate antagonist naloxone. Subsequent studies demonstrated that coadministration of μ -, but not κ - or δ -opioid antagonists also blocked self-administration behavior. We conclude that μ -receptors in the CA3 region of hippocampus may be important target sites for opioid dependence.

INTRODUCTION

The reinforcing actions of heroin and related opiate drugs are widely assumed to be mediated by specialized systems of opioid peptide neurons^{2,26,33}. The hippocampus is richly endowed with opioid peptides and receptors; in particular, enkephalins and dynorphins are contained in the mossy fiber axons which innervate pyramidal cells of the CA3 region. High densities of μ -, κ - and δ -opiate receptors are located in and near the pyramidal and granule cell layers (in rat, μ - and κ -receptors have a heavier and wider distribution in CA3, whereas δ -receptors, although distributed more uniformly in the CA fields, exhibit dense binding in CA1)^{17,37,38}.

The hippocampus is routinely associated with learning and memory functions^{18,27,53}. Some studies have shown the involvement of the hippocampus in reinforcement processes by demonstrating self-stimulation behavior in various hippocampal regions^{1,7–9,15,54,57}. Two studies have established a tentative link between hippocampal reinforcement processes and the opiate system^{15,16}. Indeed, we have reported our preliminary findings concerning dynorphin A and hippocampal reinforcement⁴⁹, as well as possible interactions with the μ -opiate receptor⁵⁰. However, definitive studies of hippocampal reinforcement in general, and its association with opiates in particular, have not been reported by others. In the present experiments, we demonstrate self-administration behavior in the CA3 region of hippocampus, using the

endogenous opiate dynorphin A as the reinforcing agent. We demonstrate further that dynorphin's reinforcing action is mainly exerted at hippocampal μ -receptors.

MATERIALS AND METHODS

Eighty-six male, Sprague–Dawley rats (Charles Rivers, Wilmington, MA), weighing 300–350 g at the time of surgery, were individually housed and maintained on a 12-h light–dark cycle (lights on at 07.00 h) with food and water freely available. Stereotaxic surgical implantation of the unilateral 23 gauge guide cannula (Plastics One, Roanoke, VA) was accomplished under sodium pentobarbital anesthesia (50 mg/kg, i.p.) with halothane as auxiliary. The tip of the guide cannula was aimed at the CA3 hippocampal region, using the following coordinates: –4.0 mm from bregma; \pm 4.0 mm lateral of midline; and –3.1 mm ventral to dura⁴². Placement of the cannula in the right or left hemisphere was randomized. The cannula was secured to the skull with stainless-steel screws and dental acrylic cement. A 14 gauge stainless-steel wire stylet, 0.5 mm longer than the guide cannula, was inserted in the cannula to maintain patency. Rats were allowed a minimum of 1 week postoperative recovery.

Drug injections were administered with the Electrolytic Micro-infusion Transducer system (EMIT) (Plastics One, Roanoke, VA). The system was devised and described by Bozarth and Wise³ and consists of a sealed reservoir containing the injection solution, a pair of platinum electrodes extending into the solution and an injection cannula which is inserted into the guide cannula aimed at the injection site. An electric current passed across the electrodes generates H_2 gas which expels very discrete volumes of fluid through the injection cannula. Currents of 200–250 μ A were used to inject drugs, with holding currents of 7–10 μ A to prevent redissolution of the H_2 gas.

Dynorphin A (Peninsula Labs, Belmont, CA), naloxone (Endo Labs, Garden City, NY), ICI 174–864 (Cambridge Biochemicals, Atlantic Beach, NY), nor-binaltorphimine and β -funaltrexamine

(RBI, Natwick, MA) were dissolved in a modified Ringer's solution (28.9 mg KCl/100 ml 0.9% saline) which also acted as vehicle. A separate control group did not have solution in the reservoir, and thus received no injections. These animals served as control for any possible mechanical effects of injections.

Rats were tested in a chamber containing a nose-poke hole equipped with an electric sensor. The nose-poke response was used because we had previously found that this behavior is more suitable than lever pressing to demonstrate electrical self-stimulation of the hippocampus¹. The testing paradigm has been published elsewhere²¹⁻²⁵. Briefly, a response at the hole triggered delivery of 100 nl injection of 5 s duration, concurrent with a 1000 Hz tone. Immediately following the injection, a 30 s 'time out' was imposed to prevent 'chaining' of injections, producing a distortion of the response measure and possible pressure-induced tissue trauma. A light over the nose-poke hole signaled availability of injections. Rats were tested over 8 h, every third day, for 3-5 test sessions. Testing occurred between 09.00 and 17.00 h. Responses for the 32 successive 15-min periods were accumulated by computer. An arbitrary 40-injection ceiling was imposed to decrease the possibility of tissue damage from excessive injections.

At the end of testing, rats were sacrificed by drug overdose (chloral hydrate, 435 mg/kg, i.p.) and transcardially perfused with physiological saline followed by 10% formalin. After decapitation, the brain was dissected out, frozen and sliced in 40- μ sections which were mounted on albumin-coated slides. After Cresyl violet staining, sections were examined to determine the injection site. Only animals with injection sites in the CA3 region of hippocampus were retained in the study (Fig. 1).

Cumulative 15-min response records were analyzed by repeated measures, multivariate analysis of variance (MANOVA) with Dunnett's *a posteriori* analysis³¹.

RESULTS

Preliminary experiments had demonstrated that self-administration of dynorphin A (DYN) over several sessions induced necrosis at the injection site despite precautions taken to avoid such an effect (Fig. 1). This complication precluded use of a more conventional self-administration paradigm involving extinction and reacquisition of self-administration behavior, and administration of several concentrations to the same animal. Thus, in these experiments, each animal received only one drug dose or combination of drugs at a single dose each, and no extinction experiments were performed.

The effective dose range for DYN was quite narrow; the highest rate of responding was manifested at the 1 pmol/100 nl injection dose and rates decreased sharply at both the 0.1 and 10 pmol/injection doses (Fig. 2). The data were expressed as the mean number of self-injections per 15 min achieved on the day of highest response for each animal in each treatment group (peak day). The use of 'peak day' data reduced the effects of such confounding factors as individual differences in learning rate, drug tolerance and tissue damage. The peak day varied from rat to rat and group to group. For the dynorphin group (1 pmol/injection), the averaged peak day was day 2. Individually, 5 animals had their best performance on day 1, 6 on day 2 and 1 on day 3. For the

vehicle and no-injection control groups, the averaged peak day was day 1. Individually, 5 of the vehicle group performed best on day 1, 4 on day 2 and 1 on day 3; in the no-injection group, 6 performed best on day 1, 2 on day 2 and 2 on day 3. Fig. 3 illustrates the changes in maximum response over test days for dynorphin, vehicle and no-injection groups. To have arbitrarily chosen a specific day for comparison between groups would have inappropriately favored one group, and even individual rats, over others. Hence the use of individual best performance or 'peak day' data for analysis.

Statistical analysis (MANOVA) revealed a significant interaction between injection and time [$F_{62,899} = 7.886$, $P < 0.001$]. A posteriori analysis showed that the rate of self-injection of DYN at 1 pmol/injection was significantly greater than for the other DYN doses or the 2 controls throughout all but the first half hour of testing ($P < 0.05$, Dunnett's). DYN 0.1 and 10 pmol/injection did not differ significantly from the vehicle control. There was a significant difference in the rate of responding between the vehicle control animals and those receiving no-injection control animals ($P < 0.05$, Dunnett's). As the vehicle control was the appropriate comparison for the drug injections, the no-injection control rates were not used in the succeeding analyses. The reinforcement



Fig. 1. Photomicrograph showing a representative cannula placement in the CA3 hippocampal region (Cresyl violet stain). The guide cannula track is marked by an open arrow. A sphere of damaged tissue (solid arrow) below the tip of the guide cannula track marks the site of drug injections. In early tests, this animal exhibited a high rate of self-administration [40 injections (maximum available) taken in the first seven 15-min time periods] for injections of dynorphin A (1 pmol/100 nl injection); in later tests, response rates decreased to vehicle levels, presumably as a result of damage to important target sites. This type of necrotic sphere was typical of animals self-injecting dynorphin A over 3 or more test sessions.

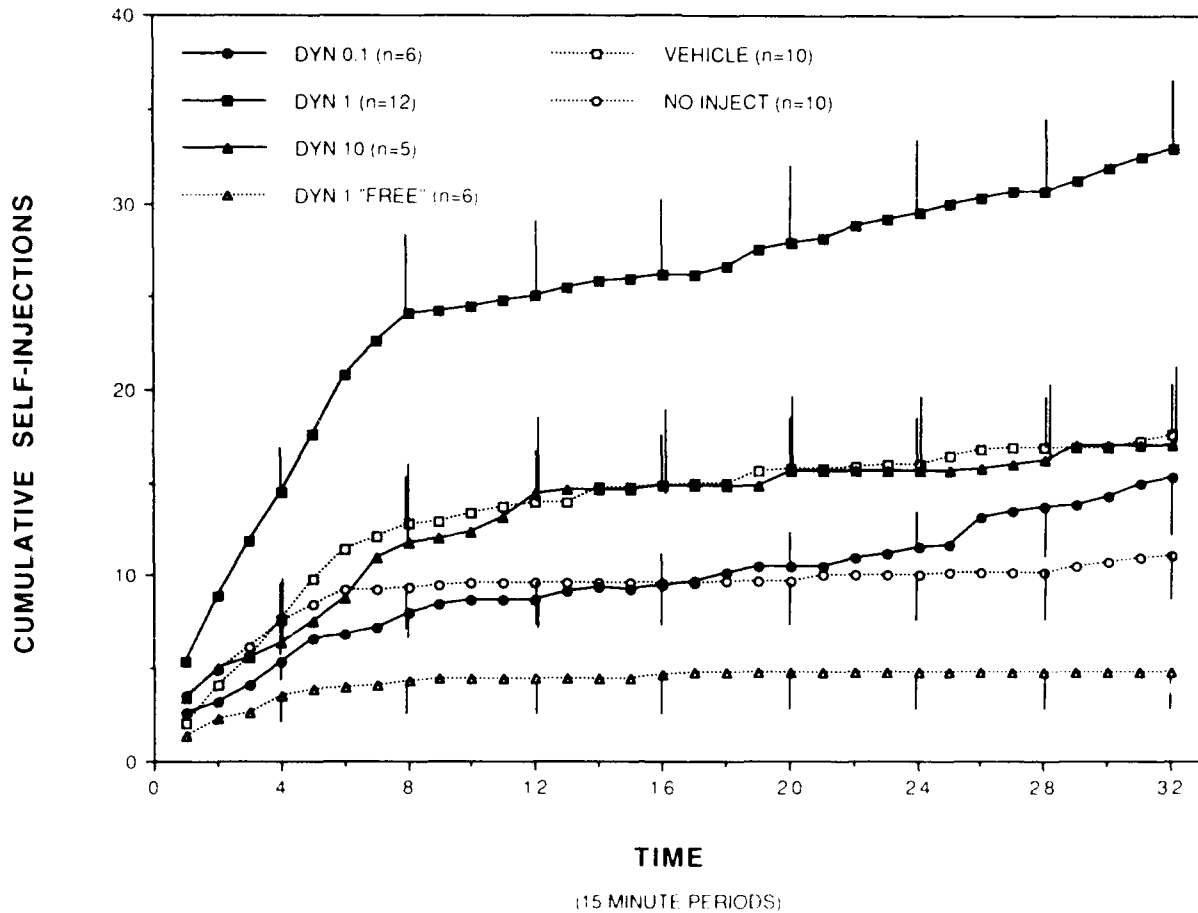


Fig. 2. Cumulative curves of CA3 hippocampal self-administration of different groups of rats given access to various concentration (0.1, 1 or 10 pmol/100 nl injection) of dynorphin A (DYN). Control groups had access to modified Ringer's solution (VEHICLE), to an empty reservoir (NO INJECTION), or were given computer-programmed ('FREE') 1-pmol injections of dynorphin A in the same temporal sequence as those earned on average by the self-injecting group. Graphed data are group means calculated by determining the daily peak response for each rat and averaging these peak scores over each treatment group. At the optimal dose of 1 pmol per injection, self-administration of dynorphin A significantly exceeded that of the other drug concentrations, as well as that of the 3 control groups. Bars represent standard error of the mean. Number of rats is indicated in parentheses.

observed with DYN injections appeared to be specific to the hippocampus since animals with cannula placements outside the hippocampus responded at rates equivalent to the control groups (Table I).

Although increases in motor activity were not observed in animals self-injecting DYN into the hippocampus, a control for non-specific behavioral stimulation was included in the study. Six additional rats received 'free' (computer-programmed) 1-pmol injections of DYN in the same temporal sequence as those earned, on average, by the self-injecting rats. A response at the nose-poke hole was counted but had no consequence. A posteriori

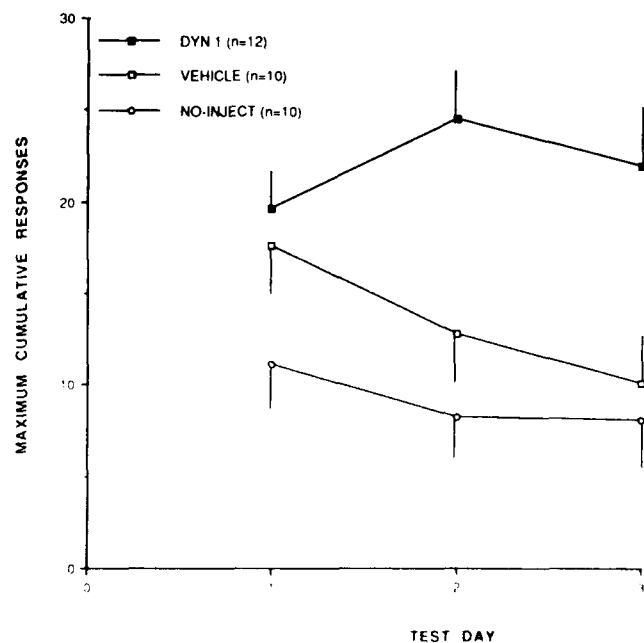


Fig. 3. Mean maximum cumulative responses (\pm S.E.M.) for animals self-administering dynorphin (1 pmol/injection), vehicle or no-injection, over the first 3 test days. Animals receiving dynorphin increased their responding from the first to the second day and dropped off slightly on the third day. Animals in both control groups (vehicle and no-injection) responded at the highest level on the first day and declined thereafter.

analysis following the significant injection-by-time interaction [$F_{93,1085} = 7.14$, $P < 0.001$] showed that response rates in the free-injection group were significantly lower than those in the self-injection group ($P < 0.05$, Dunnett's), and even fell slightly below those of the no-injection group (Fig. 2).

To test the ability of naloxone to inhibit DYN self-administration, naloxone in 2 different concentrations (100 and 500 pmol/injection) was mixed in cocktail form with the dose of DYN producing the greatest rate of self-administration, 1 pmol/injection. Naloxone was found to eliminate the initial high rate of responding seen in animals self-administering DYN alone (Fig. 4). The effect of naloxone was dose-dependent; only the 500 pmol/injection dose fully reversed the DYN-induced reinforcement. Statistical analysis comparing the naloxone cocktails with DYN alone and vehicle control rates showed a significant injection-by-time interaction [$F_{93,1085} = 2.386$, $P < 0.001$]. A posteriori analysis showed that response rates for DYN plus naloxone (500 pmol/injection) were significantly lower than rates for

TABLE I

Dynorphin self-administration scores of groups with accurate and inaccurate cannula placements in CA3

Animals were self-administering dynorphin, vehicle or no injection. Most inaccurately placed cannula were lateral to the hippocampus and terminated in the corpus callosum. One placement was in the fimbria. Rates are expressed as mean rate per hour \pm S.E.M.

| | Accurate CA3 placement | Inaccurate placement |
|-------------------|--------------------------|--------------------------|
| Dyn 1 pmol/100 nl | 32.9 \pm 3.76 (n = 12) | 13.5 \pm 0.50 (n = 2) |
| Vehicle | 17.6 \pm 2.78 (n = 10) | 17.7 \pm 11.81 (n = 3) |
| No injection | 11.1 \pm 2.38 (n = 10) | 8.7 \pm 6.68 (n = 3) |

DYN alone ($P < 0.05$, Dunnett's) and did not differ from vehicle control rates. The rates for DYN plus naloxone (100 pmol/injection) were intermediate between DYN alone and vehicle control.

To assess the effect of blockade of specific opiate receptor types on DYN self-administration, antagonists more selective than naloxone for the 3 opiate receptor

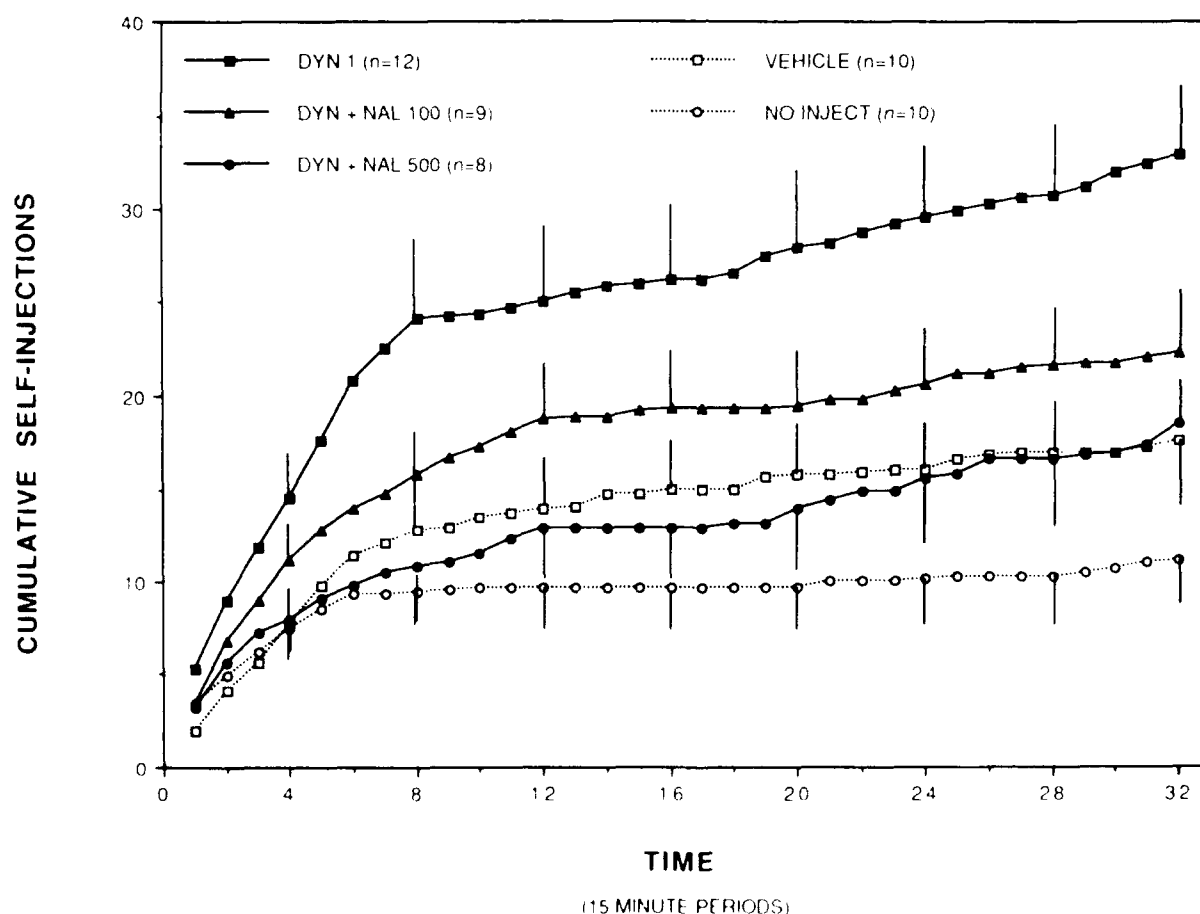


Fig. 4. Mean cumulative responses (\pm S.E.M.) for animals self-administering 1 pmol/100 nl dynorphin A or mixtures of dynorphin A (DYN) and naloxone (NAL) (100 or 500 pmol/100 nl). Vehicle and no-injection control groups self-administration rates are included for reference. Naloxone at 100 pmol per injection produced a partial reduction in dynorphin A response rates, whereas 500 pmol produced a full attenuation.

types were coadministered with DYN (1 pmol/100 nl injection). For more selective κ -receptor antagonism than naloxone confers, nor-binaltorphimine (NBNI) was used⁵²; for δ antagonism, ICI 174,864 (ICI)¹⁴, and for μ antagonism, β -funaltrexamine (β FNA)^{14,43}. Based on relative binding affinities, the dose of each of these antagonists was chosen to exert approximately the same blockade at its preferred receptor as that produced by the 500 pmol dose of naloxone (see Table II). Thus, the antagonistic action that naloxone exerts simultaneously at each of the 3 sites was reproduced separately for each receptor. The dose of each selective antagonist which was calculated to be equal to 500 pmol naloxone in blockade of the appropriate receptor type was as follows: for δ antagonism, ICI at 125 pmol/100 nl; for κ antagonism, NBNI at 5 pmol/100 nl; and for μ antagonism, β FNA at 415 pmol/100 nl.

Only coadministration of β FNA plus DYN produced self-administration rates which were different from DYN alone; coadministration of NBNI or ICI plus DYN

TABLE II

Relative binding potencies at the μ , δ and κ opiate receptors of various antagonists

The potency of naloxone (500 pmol/injection) to block each receptor type is arbitrarily assigned the value of 1. The potency of each selective antagonist, at each receptor type, is calculated relative to that of naloxone. N.A., not available.

| | μ | δ | κ |
|---------------------------|---------|----------|----------|
| Naloxone | 1 | 1 | 1 |
| β FNA ¹⁴ | 1.11 | 0.33 | N.A. |
| ICI 174,864 ¹⁴ | >0.0001 | 4 | N.A. |
| NBNI ⁵² | 0.019 | 1.5 | 100 |

produced rates similar to DYN alone (Fig. 5). Statistical analysis of both NBNI plus DYN and ICI plus DYN showed an injection-by-time interaction $\{[F_{93,1023} = 2.392, P < 0.001]$ and $[F_{93,1054} = 2.571, P < 0.001]$, respectively}. However, a posteriori analysis revealed no significant difference between DYN alone and either

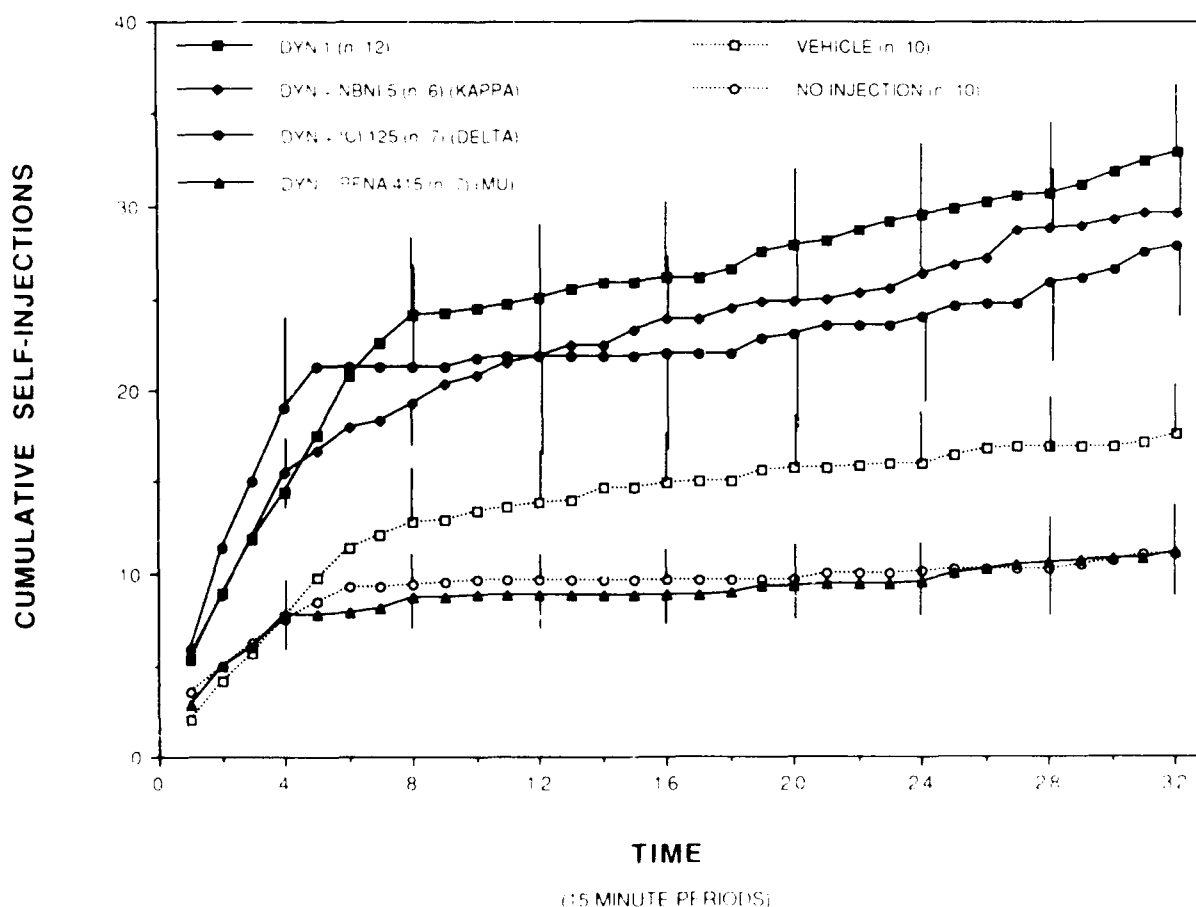


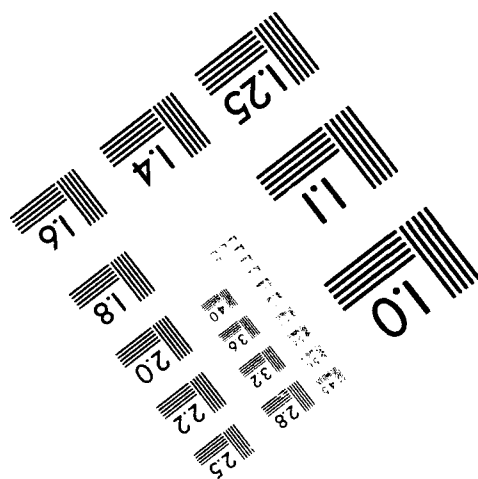
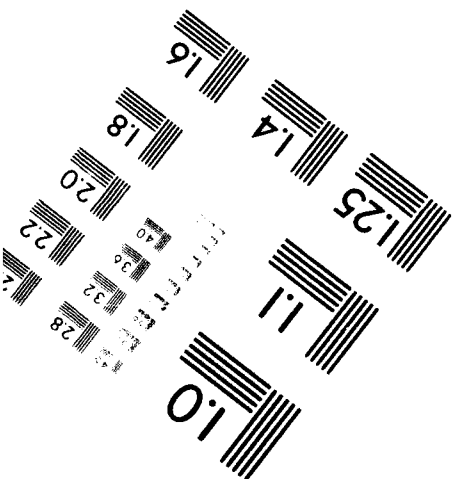
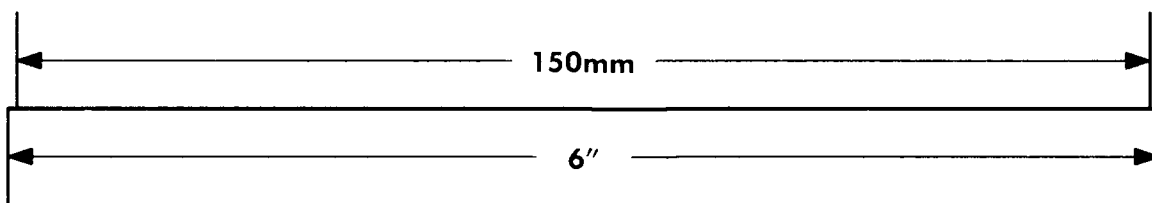
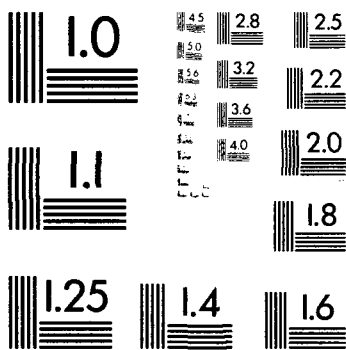
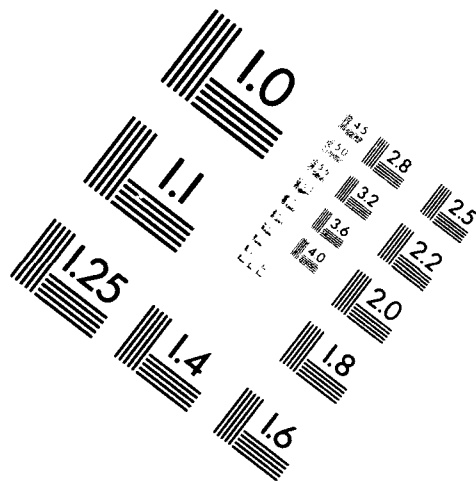
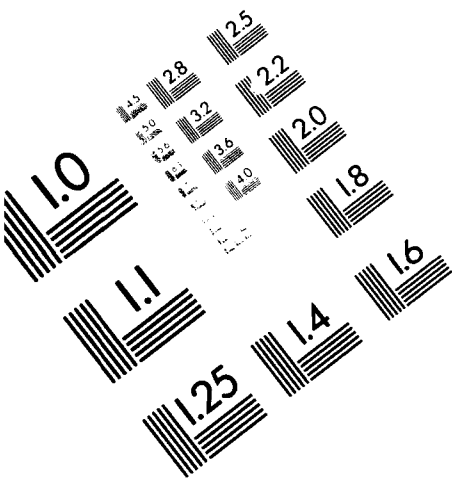
Fig. 5. Mean cumulative responses (\pm S.E.M.) for animals self-administering 1 pmol/100 nl injection dynorphin A (DYN) alone, or mixtures containing 1 pmol dynorphin A and 1 of the following: the κ antagonist NBNI (5 pmol/injection); the δ antagonist ICI (125 pmol/injection); or the μ antagonist β FNA (415 pmol/injection). Vehicle and no-injection control groups self-administration rates are included for reference. Only β FNA produced a significant reduction in dynorphin A self-administration rates, suggesting μ -opiate receptor mediation of the behavior.

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antagonist plus DYN combinations ($P > 0.05$, Dunnett's). A significant interaction between injection and time was also found with β FNA plus DYN [$F_{93,1054} = 4.698$, $P < 0.001$]. In this case, a posteriori analysis revealed that β FNA plus DYN self-administration rates were significantly less than rates for DYN alone ($P < 0.05$, Dunnett's) and were not significantly different from naloxone (500 pmol/injection) plus DYN or vehicle control rates.

DISCUSSION

These data, combined with our preliminary studies⁵⁰, are the first demonstration of the hippocampus as a site for self-administration behavior. Previous studies of self-stimulation behavior had implicated the hippocampus in the mediation of reinforcement^{1,6-9,15,39}, some indicating possible opiate involvement^{1,15}. The current investigation augments and extends these self-stimulation studies. In the present experiments, rats were shown to actively self-administer the endogenous opiate dynorphin A directly into the CA3 hippocampal region. Both a vehicle control group and a no-injection control group were included in the protocol to ascertain if some portion of the observed dynorphin reinforcement might have been produced by pressure or other mechanical effects. The small but significant increase in response rate for vehicle over no-injection controls indicated that such a mechanically-induced reinforcement was present; however, the magnitude was small compared to the reinforcement derived from the dynorphin injections.

Although the hippocampal injections of dynorphin did not appear to increase motor activity or stereotypy, it was necessary to ensure that non-specific behavioral stimulation was not responsible for the high response rates of the self-injecting rats. Accordingly, a control group was included in which rats received 'free' (computer-programmed) injections of dynorphin A at the optimally reinforcing dose. These injections were administered in the same temporal sequence as those earned by the self-injecting rats; responses made by the rats had no consequence. The rate of responding in these animals was significantly lower than the rate for the self-injecting rats and actually fell slightly below the no-injection group, thus demonstrating no behavioral activation attributable to the dynorphin injections themselves.

At the time that we first discovered dynorphin A self-administration⁵⁰, conditioned place preference evidence^{29,30} also suggested a reinforcing role for this peptide at high doses. Previously, dynorphin was not thought to be associated with reinforcement; indeed, dynorphin had been proposed as an endogenous ligand for the κ -opiate receptor^{12,13}, and the κ -receptor is

generally associated with aversion^{5,10}. In the present studies, drug-naïve rats quickly learned to self-administer dynorphin A in the CA3 region of hippocampus. At 1 pmol per injection, dynorphin A supported a high rate of self-administration which significantly exceeded the 3 control groups (vehicle, no injection and 'free' injection). The sample size of the 1 pmol group is large since a replication of the dynorphin-reinforcement effect was included, as a control for self-administration behavior, with each new set of experiments.

In accord with previous intracranial self-administration results with other drugs²⁵, the effective dose range for dynorphin A was found to be narrow; neither the 0.1 nor 10 pmol doses were effective. At the peak-response dose (1 pmol), self-administration behavior was rapidly acquired and significant differences from control rates were observed even on the first test day. The typical response pattern consisted of an early rapid rate of responding followed by a period in which the rate was much slower. Bozarth and Wise⁴ observed a similar pattern in rats self-administering morphine in the ventral tegmental area. They attributed this pattern to the differences between 'establishing and maintaining satiating drug concentrations at the reward-relevant population of receptors.' This is similar to the 'loading dose' phenomenon observed in intravenous self-administration experiments, wherein an initial rapid self-administration period is presumed to elevate blood levels to an optimal reinforcing concentration, and a slower steady-responding period serves to maintain the optimal level.

The reinforcing effects of dynorphin A were observed at a dose much lower than that required for morphine in the ventral tegmental area (130 pmol/injection). Indeed, still higher doses were required for morphine self-administration in other brain regions, such as the nucleus accumbens⁴. Even cocaine, which ranks among the most potent of chemical reinforcers, requires 50 pmol per injection to support self-administration in the medial prefrontal cortex^{22,49}. The high potency of dynorphin A as a chemical reinforcer, and its discrete localization in the mossy fiber-CA3 pathway^{17,38}, is consistent with the idea that this peptide, or an active fragment, may be a natural regulator of hippocampal reinforcement functions.

The observed self-administration of dynorphin might not have been related to opiate pharmacology since dynorphin has effects that are not mediated at opiate receptors^{28,55,56}. To determine if reinforcement with dynorphin A in the hippocampus was of opiate origin, the general opiate antagonist, naloxone³⁵ was coadministered with the reinforcing concentration of dynorphin. Naloxone dose-dependently attenuated the dynorphin reinforcement. This result suggests that dynorphin re-

inforcement is mediated via an opiate receptor. An alternate explanation, that naloxone might be suppressing learning processes in the hippocampus, is not supported by the majority of the literature. Despite the report by Messing et al.³⁶, in which systemic naloxone inhibited learning, most studies have demonstrated a facilitory role for naloxone in memory retention (for review see 19,20). Therefore, the simplest explanation of naloxone's effect in the present study is blockade of dynorphin-induced reinforcement.

Although the data indicated that dynorphin-induced reinforcement is opiate receptor mediated, naloxone's non-selectivity with respect to opiate receptor type precluded determination of specific subtype involvement with this agent. Thus, antagonists selective for the μ -, δ - and κ -opiate receptor were coadministered with dynorphin to ascertain which receptor subtype mediated the reinforcement. κ -receptor involvement was determined with nor-binaltorphimine⁵², δ -receptor involvement with ICI 174,864¹⁴ and μ -receptor involvement with β -funaltrexamine^{14,43}. The dose of the specific antagonist was selected to produce approximately the same blockade at its preferred receptor as that produced by 500 pmol/injection naloxone. Selective blockade of μ -receptors by β -funaltrexamine completely eliminated the reinforcing effects of dynorphin A, verifying our previous studies⁵¹; whereas κ -receptor or δ -receptor antagonism has only small or negligible effects. Interestingly, μ -receptor blockade reduced dynorphin responding to level below that observed with the vehicle and equal to the no-injection control. This suggests that the small, mechanically-induced reinforcement observed with the vehicle injections may be mediated, indirectly, through the μ -opiate receptor.

These results are in concert with others who have demonstrated dysphoria associated with κ -receptor activation, albeit through a possible peripheral mechanism^{5,40}. The data are also in agreement with studies which have shown dynorphin/ μ -receptor interaction by demonstrating, in vitro, that dynorphin-induced increases in electrically stimulated evoked firing of CA1 pyramidal cells are mediated at the μ -opiate receptor^{11,41}. Thus, there is precedent for the concept of dynorphin activation of μ -receptors. The current results are in accord with the fact that the powerful opiate reinforcers morphine and

heroin have high selectivity for μ -receptors and exert virtually no δ - or κ -agonist activity³³, but are not in agreement with Shippenberg et al.⁴⁵, who suggested that mediation of opiate reinforcement occurs via the δ -receptor. However, the conclusion that hippocampal opiate reinforcement occurs exclusively through the μ -receptor must be tempered by the fact that, although reversible antagonists were used for δ - and κ -receptor blockade, only the irreversible agent β FNA⁴⁴ was available for μ antagonism.

Of additional interest is the fact that the behavioral self-administration of dynorphin was predicted by in vitro hippocampal slice studies^{46,48}. In these studies, the cellular bursting patterns of spontaneously active CA3 pyramidal cells were 'reinforced' with localized applications of dynorphin A. The current findings illustrate the predictive value of the in vitro reinforcement technique.

In summary, the present work demonstrates that the hippocampus, a brain structure routinely associated with learning and memory functions, is an effective site for the self-administration of dynorphin A. This observation is consistent with clinical observations that drug-craving and compulsive drug-seeking may depend on the memory of past drug reinforcements. The reinforcing effects of dynorphin A in the hippocampus appear to be mediated, wholly or in major part, through μ -opiate receptor. Correspondence between the present observations that hippocampal applications of dynorphin A can reinforce whole-animal behavior and previous observations⁴⁸ that dynorphin A can reinforce the firing of hippocampal cells supports the hypothesis^{32,47} that individual neurons with a capacity for operant conditioning may serve as functional units for behavioral operant conditioning. If so, it is possible that such reinforceable cells are widely distributed in the brain, although an important subpopulation may be localized in the hippocampus.

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Effects of opiate antagonists and their quaternary analogues on nucleus accumbens self-stimulation

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Naloxone and naltrexone were compared with their quaternary analogues naloxone methobromide and naltrexone methobromide for efficacy in suppressing intracranial self-stimulation behavior. These quaternary analogues effectively block opiate receptors in the periphery, but since they do not readily cross the blood–brain barrier they have little effect on central receptors. Rats with electrodes in the nucleus accumbens were trained to self-stimulate in daily 60-min sessions. Naloxone (0.2, 2.0 and 20 mg/kg) and naltrexone (20 mg/kg) potently suppressed self-stimulation behavior. In contrast, neither naloxone methobromide (0.2 and 20 mg/kg) nor naltrexone methobromide (20 mg/kg) had any significant effects on this behavior. These results suggest that blockade of peripheral opiate receptors alone is insufficient to suppress self-stimulation, and therefore support the idea that opiate antagonists suppress self-stimulation by blockade of central receptors that mediate reinforcement.

INTRODUCTION

The idea that endogenous opioids might play an important role in the mediation of reinforcement has been supported by the observation that opiate receptor antagonists suppress self-stimulation behavior^{1,8,17,30,31,34}. According to this idea, animals respond in self-stimulation for electrically-released endogenous opioids; blockade of opiate receptors prevents the reinforcing actions of these transmitters, thereby suppressing response rates or increasing threshold for self-stimulation. However, since self-stimulation behavior is sensitive to effects unrelated to reinforcement, some investigators have suggested

that the suppressive effects of opiate antagonists might be due to non-specific actions, such as impaired movement^{14,27,35}.

A critical issue yet to be resolved about the suppressive effects of opiate antagonists in self-stimulation pertains to their site of action. It is possible that these compounds non-specifically affect self-stimulation by acting at opiate receptors in the periphery. Opiate receptors are known to exist in several peripheral tissues including vas deferens, adrenal medulla and gut¹⁶. Action at one or more of these sites might cause unpleasant side-effects and lead to the observed suppression of self-stimulation. On the other hand, in order to conclude that opiate antagonists suppress self-

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stimulation by specifically interfering with brain mechanisms of reinforcement, a central site of action must be demonstrated.

Quaternary analogues of the opiate receptor antagonists naloxone and naltrexone have been developed which retain the ability to block opiate receptors, yet do not readily cross the blood-brain barrier^{3,4,29,39}. Peripheral administration of these compounds therefore blocks peripheral opiate receptor sites without affecting receptors in the central nervous system. Use of these compounds has helped to demonstrate a central site of action for the discriminative stimulus properties of opiate agonists and antagonists³⁹, for the suppressive effects of naloxone on fluid consumption^{5,11} and stimulation-induced feeding⁶, and for the reinforcing effects of heroin^{21,38}.

The present study compared the effects of naloxone-HCl and naltrexone-HCl with their quaternary derivatives naloxone methobromide and naltrexone methobromide on self-stimulation of the nucleus accumbens. The accumbens is rich in both opioid peptides and opiate receptors, and self-stimulation of this nucleus is sensitive to suppression by opiate antagonists^{7,17,33,36,37}. In addition, this nucleus appears to be important in mediating the reinforcing effects of opioids^{15,24,32,38,41}. If the quaternary antagonists suppressed self-stimulation in a manner similar to the tertiary forms, then one would conclude that suppression of self-stimulation by opiate antagonists is due to peripheral side-effects of these drugs and not due to blockade of reinforcement. On the other hand, a lack of effect of the quaternary analogues would suggest a central site of action for opiate antagonists, consistent with the hypothesis that these drugs act by interfering with reinforcement.

MATERIALS AND METHODS

Animals

Experimentally naive male albino Sprague-Dawley rats (Charles River) were used. The animals weighed 290–400 g at the time of surgery, and were individually housed on a 12-h light/dark cycle with food and water available ad libitum.

Surgery

Rats were anesthetized with sodium pentobarbital (45 mg/kg i.p.) and stereotactically implanted with bipolar electrodes (Plastic Products MS 303/8) aimed at the nucleus accumbens (coordinates, skull level with horizontal: A-P + 2.0 mm from bregma; L 1.2 mm from midline; D-V – 6.0 from the brain surface). Electrodes were attached to the skull using stainless steel screws and dental cement.

Apparatus

Twelve chambers (28 × 25 × 30 cm high) each containing a lever (3.8 × 1.3 × 1.5 cm) mounted on the rear wall 4 cm above the grid floor were used for self-stimulation experiments. Chambers were constructed of Plexiglas with black rear and side walls, and clear door and ceiling. A light located above the lever remained on when the stimulation was available. Self-stimulation chambers were individually housed in sound-insulated compartments with white noise. A single lever-press (10 g force) delivered a 150-ms train of electrical brain stimulation consisting of monophasic rectangular pulses of 0.2 ms duration presented at 100 Hz through an isolation transformer. Electrical connection through a commutator allowed the rat free movement in the chamber at all times. Lever-presses were automatically counted and recorded at 5-min intervals by a computer interfaced with the chambers via a BRS-LVE Interact system. In addition, cumulative recorders continuously monitored responding throughout the session.

Procedure

Following at least one week recovery from surgery, animals were trained to self-stimulate at 350 μ A current intensity in 60-min sessions. After stable response rates were achieved at this intensity, a descending rate-intensity function was determined for each rat to identify the lowest current that would maintain stable responding. This was achieved in a single self-stimulation session as follows: rats began responding in self-stimulation at 350 μ A current intensity as normal. Current intensity was readjusted downward by 25–50 μ A every 5 min, until responding became

disrupted or intermittent. At this point, current was adjusted up and down around this intensity to establish the lowest value that would maintain stable responding. This current intensity was identified for each animal as the 'threshold' or 'baseline' current and remained at the new value for the duration of the experiment. Drug tests began after response rates restabilized at the new 'baseline' current intensities. Since drug effects are more pronounced at threshold current than at maximal current, use of these low baselines represents a more sensitive assessment of the reinforcement mechanisms underlying the self-stimulation behavior than use of higher current intensities⁴².

Animals were tested in self-stimulation 5 days per week. Animals received no treatment on days one, two and five; day three served as saline control session; drug injections were performed on day four. Injections consisted of naloxone-HCl (0.2, 2.0 or 20 mg/kg) or naloxone methobromide (0.2 or 20 mg/kg) administered in a random order for individual animals, followed by naltrexone-HCl (20 mg/kg) or naltrexone methobromide (20 mg/kg s.c.) administered in random order. If response rate changed by more than 10% during the saline control session, no drugs were administered that week. Drugs were dissolved in sterile saline and administered subcutaneously in a volume of 1 ml/kg immediately prior to the experimental session.

Histological analysis

Upon completion of experiments, animals were given an overdose of chloral hydrate and perfused intracardially with saline followed by 10% formalin. Brains were removed, frozen, and sectioned at 40 μ m. Electrode placements were verified using the atlas of König and Klippel²⁰.

Data analysis

The number of lever presses during the final 45 min of a drug session was compared to the final 45 min of the preceding saline control session and expressed as mean percent of control. Paired *t*-test analysis assessed whether experimental effects were different from the control session.

RESULTS

Of the 12 animals implanted with electrodes, one lost the implant prior to drug injections and was therefore not used in this study. Histological analysis revealed that the electrode tips for 10 out of the 11 remaining animals were located in the anterior half of the nucleus accumbens²⁰ (see Fig. 1). The eleventh animal lost its electrode prior to perfusion and the tip was therefore not localized. However, due to the consistency in the remaining 10 placements, this electrode was assumed to be similarly placed. Mean baseline response rate at the beginning of experiments was 1109 ± 135 lever-presses/45 min, and mean baseline current intensity 136 ± 11 μ A. Rate-intensity sessions revealed that response rates were dependent on current intensity — reduction in current resulted in an intensity-related decrease in responding (Table I).

Naloxone-HCl dose-dependently suppressed self-stimulation of the nucleus accumbens (Fig. 2). Significant effects were observed at all doses tested: 0.2 mg/kg ($83.0 \pm 6.5\%$ of saline control, $n = 11$, $P < 0.01$), 2.0 mg/kg ($61.7 \pm 14.1\%$ of saline control, $n = 8$, $P < 0.05$), and 20 mg/kg ($35.0 \pm 11.9\%$ of control, $n = 8$, $P < 0.01$). In contrast, neither 0.2 mg/kg of naloxone methobromide ($99.3 \pm 3.5\%$ of control, $n = 11$, n.s.) nor 20 mg/kg ($95.4 \pm 2.9\%$ of control, $n = 8$, n.s.) had any effect on the behavior.

TABLE I

Response rate is dependent on current intensity

Data are from the rate-intensity sessions for the 11 animals in this experiment. Number of animals tested at each intensity is identified in parentheses. Note that response-rate decreases as current is decreased.

| Current intensity (μ A) | Mean number of responses 5 min |
|------------------------------|--------------------------------|
| 350 | 168 (11) |
| 300 | 174 (11) |
| 250 | 156 (10) |
| 200 | 105 (9) |
| 150 | 74 (9) |
| 100 | 33 (8) |

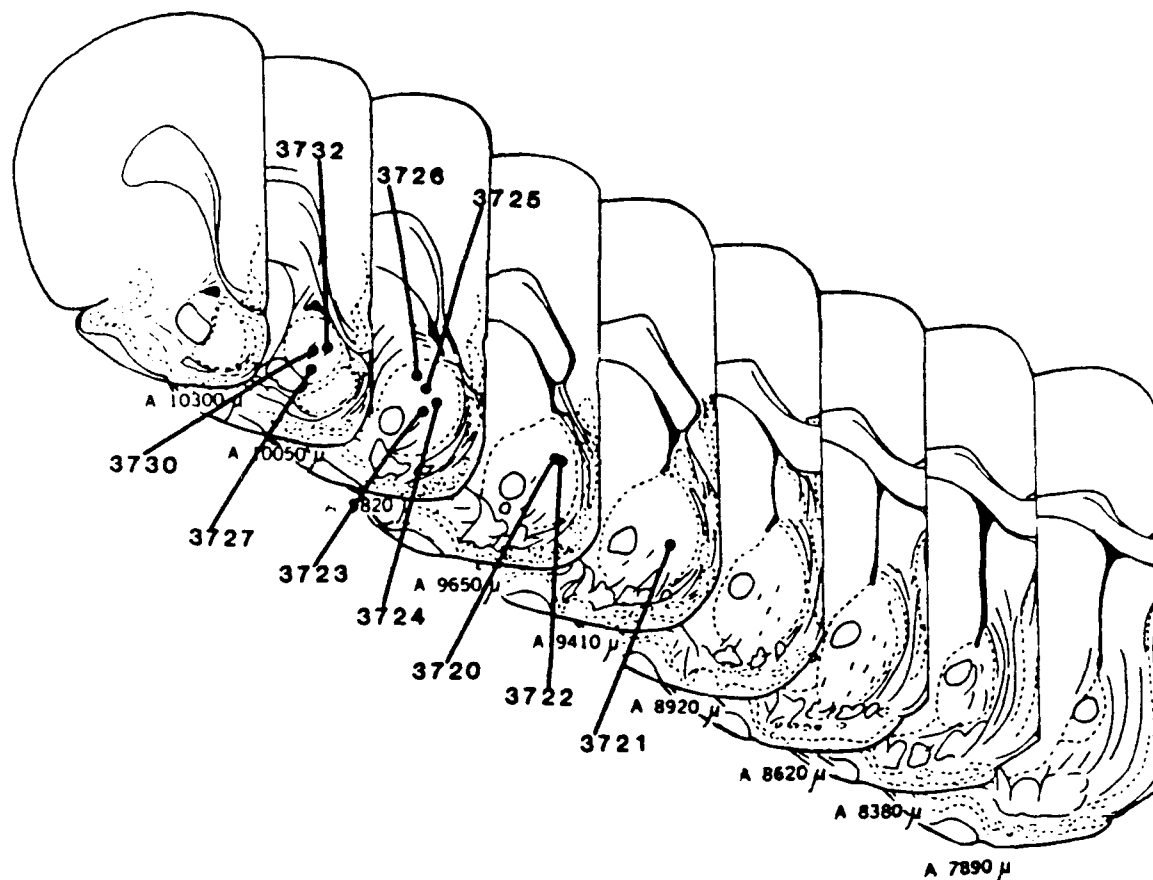


Fig. 1. Summary diagram of electrode placements. Electrode tips are indicated by filled circles on representative brain sections from the atlas of König and Klippel²⁰. All electrodes are in the anterior half of the nucleus accumbens.

Similar effects were observed for naltrexone-HCl and naltrexone methobromide (Fig. 3). While 20 mg/kg of naltrexone-HCl potently suppressed self-stimulation ($42.6 \pm 15.7\%$ of control, $n = 5$, $P < 0.05$), the same dose of naltrexone methobromide had no effect on this behavior ($95.7 \pm 4.1\%$ of control, $n = 5$, n.s.).

DISCUSSION

In the present study, the tertiary opiate receptor antagonists naloxone-HCl and naltrexone-HCl were observed to potently suppress self-stimulation of the nucleus accumbens following peripheral administration, a finding consistent with previous studies^{17,33,36,37}. In contrast, the quaternary opiate antagonists naloxone methobromide

and naltrexone methobromide, which do not readily cross the blood-brain barrier, had no significant effects on this behavior. If blockade of peripheral receptors was responsible for the rate-decreasing effects of opiate antagonists on self-stimulation, the quaternary antagonists would have been expected to have actions similar to the tertiary compounds. The results lead us to conclude that opiate antagonists suppress self-stimulation by blocking opiate receptors in the central nervous system rather than in the periphery. A possible alternative explanation for the present results is that blockade of opiate receptors in both the periphery and the central nervous system is necessary to suppress self-stimulation. Thus, peripheral blockade may be necessary but not sufficient to suppress this behavior. Although we consider this unlikely, future studies utilizing cen-

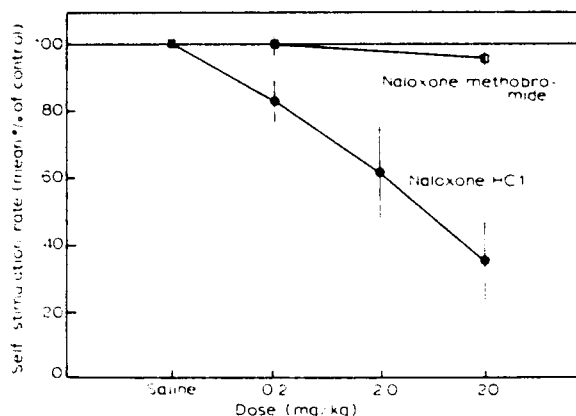


Fig. 2. Effects of naloxone-HCl and naloxone methobromide on self-stimulation of the nucleus accumbens. Data points represent mean percent control \pm standard error for the final 45 min of the session. Significant effects were observed at all doses of naloxone-HCl tested: 0.2 mg/kg ($83.0 \pm 6.5\%$ of saline control, $n = 11$, $P < 0.01$), 2.0 mg/kg ($61.7 \pm 14.1\%$ of saline control, $n = 8$, $P < 0.05$), and 20 mg/kg ($35.0 \pm 11.9\%$ of control, $n = 8$, $P < 0.01$). In contrast, neither 0.2 mg/kg of naloxone methobromide ($99.3 \pm 3.5\%$ of control, $n = 11$, n.s.) nor 20 mg/kg ($95.4 \pm 2.9\%$ of control, $n = 8$, n.s.) had any effects on the behavior.

tral administration of quaternary antagonists will help to distinguish between these possibilities.

The present results support and extend the recent findings of Schaefer and Michael³¹ on comparisons between opiate antagonists and their quaternary derivatives on self-stimulation behavior. These authors reported that animals with electrodes in the midbrain central gray area, responding for self-stimulation on a fixed ratio: 20 schedule, were suppressed by naloxone-HCl and naltrexone-HCl but not by naloxone methobromide or naltrexone methobromide. The present results extend these findings to animals responding on a continuous reinforcement schedule with electrodes in the nucleus accumbens. It therefore appears that the effects of opiate antagonists and their quaternary derivatives on self-stimulation are similar under a variety of conditions.

It is important to note that significant potency differences have been observed between the tertiary opiate antagonists and their quaternary analogues for blockade of opiate receptors. Naloxone-HCl has been observed to be 10–28

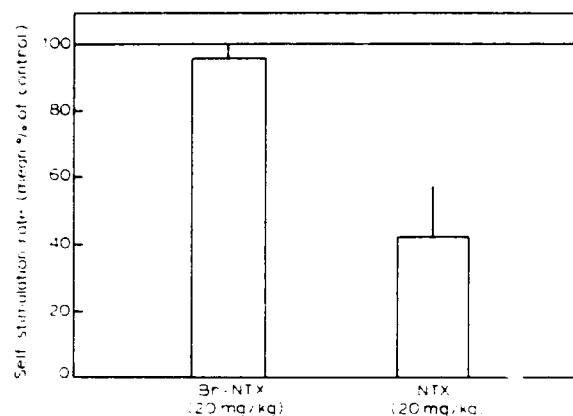


Fig. 3. Effects of naltrexone-HCl (NTX) and naltrexone methobromide (BrNTX) on self-stimulation of the nucleus accumbens. Mean percent control \pm standard error. NTX significantly suppressed this behavior ($42.6 \pm 15.7\%$ of control, $n = 5$, $P < 0.05$); BrNTX had no significant effects ($95.7 \pm 4.1\%$ of control, $n = 5$, n.s.).

times as potent as naloxone methobromide^{22,39,40} and naltrexone-HCl 50–77 times as potent as naltrexone methobromide²² in displacing [³H]etorphine from rat brain opiate receptors. In addition, naloxone-HCl has been observed to be approximately 28 times as potent as naloxone methobromide^{19,40}, and naltrexone-HCl 26–39 times as potent as naltrexone methobromide^{39,40} for antagonizing the depressant effects of morphine on electrically stimulated contractions of the guinea pig ileum in vitro. Lack of effects of the quaternary analogues might therefore have resulted from the decreased potency relative to the tertiary antagonists. In the present study, however, 0.2 mg/kg naloxone-HCl was observed to significantly suppress self-stimulation behavior, whereas 100 times this dose of either quaternary analogue failed to affect the behavior. Therefore, even the most conservative estimates of potency difference cannot explain the lack of effects of the quaternary analogues. Furthermore, the fact that quaternary antagonists have significant effects in the periphery in vivo at doses similar to those used in the present study suggests that peripheral opiate receptors are indeed being blocked by these compounds^{3,6,26}.

Quaternary derivatives of opiate antagonists have proven useful in distinguishing the central

actions of opiate antagonists from peripheral effects⁴. Particularly relevant to the present study are demonstrations that the suppressive effects of opiate antagonists on drinking^{5,11,25} and feeding^{6,23} are centrally mediated, since the role of endogenous opioids in these behaviors may be to mediate reinforcement^{6,9,10,25}. Also relevant to the present study is the demonstration that the reinforcing effects of self-administered opiates are centrally mediated²¹, and that opiate receptors in the nucleus accumbens are critical for these actions³⁸.

There has been considerable debate over whether opiate antagonists suppress brain-stimulation reward by specifically blocking the reinforcing actions of stimulation-released endogenous opioids^{1,8,17,30,31,34}, or by non-specific effects on the ability of the animal to respond^{14,27,35}. The present results' suggesting that opiate antagonists suppress self-stimulation at central sites, is essential for the argument that opiate antagonists act by specifically blocking reinforcement. However, since these compounds can also have non-specific effects in the central nervous system, the results do not confirm a specific blockade of reinforcement. On the other hand, several studies do suggest that opiate antagonists specifically block the reinforcing effects of stimulation-released endogenous opioids. (1) Doses required to suppress self-stimulation are substantially lower than those required to suppress locomotor activity, suggesting that motor incapacity is not responsible for the rate-decreasing effects of these drugs. In the present study, for example, significant suppression of self-stimulation was observed at 0.2 mg/kg of naloxone, the lowest dose examined. In contrast, suppression of locomotor activity requires doses of 10 mg/kg or greater¹². (2) Further evidence that locomotor effects are not involved is the demonstration that nose-poking for self-stimulation, which requires less motor output than lever-pressing, is nevertheless suppressed as strongly as the latter by opiate antagonists². (3) The demonstration that opiate antagonists produce an extinction-like response decrement pattern on self-stimulation offers strong support for the suggestion that these compounds affect the behavior by specifically

blocking reinforcement³⁷. (4) Differences between the effects of these drugs on stimulation-produced analgesia and brain-stimulation reward suggest that blockade of reinforcement, and not increased aversion, is the mechanism by which they suppress self-stimulation¹⁸.

In summary, the present results support and extend previous findings that opiate antagonists suppress self-stimulation by actions in the central nervous system, rather than in the periphery. These results are consistent with the suggestion that naloxone and naltrexone suppress self-stimulation by specific effects on reinforcement, and add to the increasing evidence that endogenous opioids may play an important role in the mediation of reinforcement^{1,9,10,13,28,34}.

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Opiate antagonists and self-stimulation: extinction-like response patterns suggest selective reward deficit*

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Key words: Naloxone; Naltrexone; Self-stimulation; Reinforcement; Extinction; Endorphin

The present study investigated the response decrement patterns produced by opiate antagonists on intracranial self-stimulation behavior, in order to determine if these drugs affect the reinforcement value of the stimulation or interfere with the ability of the animal to respond. Male rats lever-pressed in 60-min sessions on a continuous reinforcement schedule for self-stimulation of the nucleus accumbens. Naloxone (2.0 and 20 mg/kg) and naltrexone (2.0 and 20 mg/kg) suppressed self-stimulation only after a significant delay, in an extinction-like response decrement pattern, mimicking the effects of reductions in current intensity (75% and 50% of baseline). The increasing behavioral effects characteristic of the extinction pattern were observed despite the fact that testing began after the time point at which maximal suppression of self-stimulation occurs with these drugs, and when brain concentrations of these drugs were declining. Since normal responding was observed for several minutes after the beginning of the session, the results may explain why long sessions are necessary to observe suppression of self-stimulation by opiate antagonists. The extinction-like pattern produced by these drugs suggests that opiate antagonists suppress self-stimulation by reducing the reinforcement value of the stimulation, rather than by interfering with the ability of the animal to respond. These findings are consistent with a role for endogenous opioid peptides in brain stimulation reward.

INTRODUCTION

Belluzzi and Stein in 1977³ obtained the first evidence that endogenous opioids might be involved in positive affect and reinforcement. Among their findings was the observation that naloxone, a potent and selective opiate receptor antagonist, suppressed self-stimulation of opioid-rich brain regions. They reasoned that opioid peptides, released from neurons by stimulation at the tip of the electrode were responsible, at least in part, for the reinforcing effects of the stimulation. Naloxone, by blocking opiate receptors, prevented the reinforcing action of these peptides, and led to the observed decreases in responding. The findings of Belluzzi and Stein were soon challenged, however, as van der Kooy, LePiane and Phillips⁷⁴ almost concomitantly reported that naloxone had no effects on self-stimulation, leading these investigators to conclude that endoge-

nous opioids were not involved in brain-stimulation reward. The effects of opiate antagonists on self-stimulation behavior have since been widely studied, yielding divergent results and various interpretations from different investigators.

A survey of published results reveals 31 studies demonstrating a suppressive action of opiate antagonists on self-stimulation behavior and 20 studies observing no effects (Table I). These differences have resulted in considerable controversy regarding the effects of opiate antagonists on self-stimulation – firstly, over whether these drugs do in fact have significant effects, and secondly, over the interpretation of effects when they are observed. In regard to the first question, it is apparent from examining these studies that different self-stimulation procedures can yield different results from opiate antagonists. West, Schaefer and Michael⁸⁰ have suggested several reasons for these differences, including (1)

* A preliminary report of this work was presented at the 1984 Society for Neuroscience Meeting in Anaheim, California⁷².

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TABLE 1A

Studies observing suppression of self-stimulation with opiate antagonists

Published studies examining the effects of opiate antagonists on brain-stimulation reward. * Identifies studies examining only insensitive electrode implant sites (hypothalamus or medial forebrain bundle). ** Identify studies using extremely short test sessions (10 min or less). Amyg = amygdala; Cd = caudate; CG = central gray; DG = dentate gyrus; DT = dorsal tegmentum; LC = locus coeruleus; LH = lateral hypothalamus; MEC = medial entorhinal cortex; MFB = medial forebrain bundle; NAcc = nucleus accumbens; NPnt = nucleus paratenalis; PAG = periaqueductal gray; PFC = prefrontal cortex; PH = posterior hypothalamus; PVC = periventricular gray; SN = substantia nigra; Sp = septum; VTA = ventral tegmental area.

| Investigators | Implant site | Length of session |
|--------------------------------------|-------------------|----------------------|
| Belluzzi and Stein ⁷ | CG | 60 min |
| Gimino et al. ²³ | Various Sites | not given |
| Stapleton et al. ⁶⁵ | PAG, NAcc, LH | ** 3 min |
| Stapleton et al. ⁶⁵ | PAG, NAcc, SN, LH | 60 min |
| Stein and Belluzzi ⁶⁸ | MFB, LC, Sp, NPnt | 60 min |
| Cruz-Morales and Reid ¹² | Amyg | 60 min |
| Lewis ⁴⁰ | MFB, VTA | 15 min |
| Katz ³⁶ | NAcc | 5 days |
| Lewis ⁴¹ | MFB, VTA | not given |
| Schaefer and Michael ⁵⁸ | LH, CG | 60 min |
| Franklin and Robertson ²⁰ | LH, DT, PFC | 60 min |
| Glick et al. ²⁴ | * LH | 50 min |
| Schaefer and Michael ⁵⁹ | CG | not given |
| Van Wolfswinkel et al. ⁷⁷ | VTA | 28 min |
| Bermudez-Rattoni et al. ⁵ | * LH | 60 min |
| Loughlin et al. ⁴⁴ | LC, PVC | 60 min |
| Trujillo et al. ⁷¹ | NAcc | 60 min |
| West et al. ⁸⁰ | CG | 20 min |
| Freedman and Pangborn ²¹ | MFB, CG | 30 min |
| Kelsey et al. ³⁷ | CG | 80 min |
| Collier et al. ⁹ | DG | 15 min |
| Trujillo et al. ⁷² | NAcc | 60 min |
| Belluzzi et al. ⁴ | NAcc | 60 min |
| Ichitani et al. ³³ | * LH | ** 2 × 10 min |
| Schaefer et al. ⁶⁰ | CG | 45 min |
| Stein ⁶⁶ | * LH | 180 min |
| Van Wolfswinkel et al. ⁷⁶ | VTA | 25 min |
| Van Wolfswinkel et al. ⁷⁸ | VTA | 15 min (acquisition) |
| West and Wise ⁸¹ | NAcc, LH, VTA | not given |
| Reymann et al. ⁵⁵ | MEC | 30 min |
| Ichitani and Iwasaki ³² | CG | 160 min |
| Schaefer and Michael ⁶¹ | CG | 30 min |

mean = 51.7 ± 7.7 min
(not incl. 5 day session)
median = 60 min

TABLE 1B

Studies not observing suppression of self-stimulation with opiate antagonists

See for legend Table 1A.

| Investigators | Implant site | Length of session |
|-----------------------------------|-----------------|-------------------|
| Wauquier et al. ⁷⁹ | * LH | 30 min |
| Pert ⁵² | * PH | 30 min |
| Holtzman ³¹ | * LH | 75 min |
| Bozarth and Reid ⁶ | * LH | ** 2 min |
| Goldstein and Malik ²⁶ | * LH | 30 min |
| Van der Kooy et al. ⁷⁴ | * LH, Cd | 30 min |
| Lorens and Sainati ⁴³ | * LH | ** 10 min |
| Wiebel and Wolf ⁸² | * LH | 60 min |
| Esposito et al. ¹⁴ | * LH | discrete trial |
| Stillwell et al. ⁶⁹ | LH, PAG | 80 min |
| Nazarro et al. ⁴⁷ | SN, VTA | ** 10 min |
| Esposito et al. ¹⁵ | * LH | discrete trial |
| Nazarro et al. ⁴⁸ | SN, VTA | ** 10 min |
| Perry et al. ⁵¹ | LH, VTA | discrete trial |
| Seeger et al. ⁶³ | VTA | 15 min |
| Leith ³⁹ | * MFB | 15 min |
| Potter et al. ⁵³ | * LH (hamsters) | 4 days |
| Carr and Simon ⁷ | * LH | not given |
| Schenk et al. ⁶² | * LH | 60 min |
| Kamata et al. ³⁵ | VTA | not given |

mean = 32.6 ± 6.9 min
(not incl. 4 day session)
median = 30 min

length of test session, (2) schedule of reinforcement, (3) electrode implant site, and (4) stimulation parameters. Electrode placement and length of test session appear to be particularly important. While lateral hypothalamic (LH) or medial forebrain bundle (MFB) electrode sites are relatively insensitive to the effects of opiate antagonists, other sites such as central gray or ventral tegmental area appear more sensitive^{21,40,41,58,68} (but also see ref. 20). In regard to the length of the test session, long sessions appear important for observing significant effects of these drugs on self-stimulation. While 15 of the 31 studies that observed suppression used sessions of 60 min or greater, only 5 of the 20 studies that failed to find suppression used sessions of this length. Additionally, positive studies had a mean session length of over 50 min, while negative studies averaged just over 30 min in duration. Analysis of the 20 negative studies with respect to electrode implant site and length of test session, reveals that 12 of these studies

examined insensitive brain sites, two used exceedingly short test sessions (10 min or less), and two used a combination of insensitive site and short session. Therefore, on the basis of these two factors alone, negative findings could have been predicted in 16 of the 20 negative studies. In contrast, negative findings would have been predicted in only 5 of the 31 positive studies based on the same criteria. This survey makes it apparent that, if sensitive methods are used, opiate antagonists do indeed have significant suppressant effects on self-stimulation.

A second important issue pertinent to the study of opiate antagonists on self-stimulation regards the interpretation of the suppressant effects. While drugs that interfere with reinforcement will suppress self-stimulation behavior, compounds that impair the ability of an animal to respond will also suppress behavior in this experiment. Therefore, while some investigators believe that suppression of self-stimulation by opiate antagonists reflects a selective disruption of reinforcement^{3,9,36,58,66}, others suspect that these effects might be due to a non-specific performance deficit, such as sedation or motor debilitation^{20,51,69}. Despite these disagreements, few studies have addressed the reward versus performance problem experimentally. The present study is an attempt to distinguish between reinforcement and performance as possible explanations of the suppression of self-stimulation by opiate receptor antagonists.

Liebman⁴² has provided an exhaustive review of methods available for distinguishing reward-related effects of drugs from non-specific performance deficits in self-stimulation. Examination of response patterns is one such method. When the reinforcer is withdrawn from an animal responding in an operant task, a characteristic extinction pattern of responding is observed. This pattern is characterized by initial normal (or even facilitated) rates of responding, followed soon by decreases. In self-stimulation, if a drug acts by selectively blocking the reinforcement value of the stimulation, an extinction-like response pattern will be observed. Alternatively, when a drug non-specifically interferes with responding, decrements occur from the beginning of the session, without initial normal rates of response⁴². Careful examination of response patterns has proven useful in distinguishing between reward and perfor-

mance effects of catecholamine antagonists in self-stimulation^{17-19,22}.

In the present study the response decrement patterns produced by naloxone and naltrexone on self-stimulation were examined, in order to determine if opiate antagonists suppress this behavior by reducing the reinforcement value of the stimulation or by interfering with the ability of the animal to respond. The brain site examined was the nucleus accumbens. This site was chosen because it is rich in both opioid peptides and opiate receptors, and self-stimulation of this nucleus is sensitive to suppression by opiate antagonists^{8,36,65,71,72}. Furthermore, this nucleus appears to be important in mediating the reinforcing effects of opioids^{25,50,64,73,75}.

MATERIALS AND METHODS

Animals

Experimentally naive male albino Sprague-Dawley rats (Charles River) were used in these experiments. The animals weighed 320–425 g at the time of surgery, and were individually housed on a 12-h light/dark cycle with food and water available ad lib.

Surgery

Thirteen rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and stereotactically implanted with bipolar electrodes (Plastic Products MS 303/8) aimed at the nucleus accumbens (coordinates, top of skull level with horizontal plane: A-P +2.0 mm from bregma; Lat. 1.2–1.3 mm from midline; D-V –6.0 from the brain surface). Electrodes were attached to the skull using stainless steel screws and neuroplastic cement.

Apparatus

Twelve chambers (28 × 25 × 30 cm high) each containing a lever (3.8 × 1.3 × 1.5 cm) mounted on the rear wall 4 cm above the grid floor were used for self-stimulation experiments. Chambers were constructed of Plexiglas with black rear and side walls, and clear door and ceiling. A light located above the lever remained on when the stimulation was available. Self-stimulation chambers were individually housed in sound-insulated compartments with white noise. A single lever-press (10-g force) delivered a

150 ms train of electrical brain stimulation consisting of monophasic rectangular pulses of 0.2 ms duration presented at 100 pulses per second through an isolation transformer. Electrical connection through a commutator allowed the rat free movement in the chamber at all times. The number of lever presses were automatically counted and recorded at 5-min intervals by a Data General Nova 4 computer interfaced with the chambers via a BRS-LVE Interact System. In addition, cumulative recorders continuously monitored responding throughout the session.

Procedure

Following at least one week recovery from surgery, animals were trained to self-stimulate at 350 μ A current intensity in 60-min sessions. After stable response rates were achieved at this intensity, a descending rate-intensity function was determined for each rat to identify the lowest current that would maintain stable responding. This was achieved in a single self-stimulation session as follows: rats began responding in self-stimulation at 350 μ A current intensity as normal. Current intensity was readjusted downward by 25–50 μ A every 5 min, until responding became disrupted or intermittent. At this point, current was adjusted up and down around this intensity in order to establish the lowest value that would maintain stable responding. This current intensity was identified for each animal as the 'threshold' or 'baseline' current. Current remained at the new value for the remainder of the animal's history, except when altered experimentally. Experiments began after response rates restabilized at the new 'baseline' current intensities. Since drug effects are more pronounced at threshold current than at maximal current, use of these low baselines represents a more sensitive assessment of the reinforcement mechanisms underlying the self-stimulation behavior than use of higher current intensities⁷⁷. To assure that experimenter-dependent priming effects did not contribute to the response patterns observed, no priming stimulations were administered in any remaining sessions; i.e. the rats initiated lever-pressing on their own, without inducement by the experimenter.

During the course of experiments animals were run in self-stimulation 5 days per week. On days 1,

2 and 5, animals received no treatment; day 3 served as experimental control session; experimental manipulations were performed on day 4. Control sessions consisted of saline administration for drug experiments, and baseline current intensity for current manipulations. If response rate changed by more than 10% during a control session, no experimental manipulation was performed that week. Experimental manipulations were separated by at least 7 days and included: naloxone HCl (2.0 mg/kg) administered either immediately prior or 30 min prior to the beginning of the test session, followed one week later by the converse: naloxone HCl (20 mg/kg) administered either immediately prior or 30 min prior to the test session, followed one week later by the converse: naltrexone HCl (2.0 mg/kg) administered 30 min prior to the test session; naltrexone HCl (20 mg/kg) administered 30 min prior to the test session; 50% current intensity; 75% current intensity. Drugs were dissolved in sterile saline and administered subcutaneously (s.c.) in a volume of 1 ml/kg.

The administration of naloxone 30 min prior to the session assured that the response pattern observed was not due to drug-induction effects; i.e. increasing drug availability as the session progressed. Previous studies have shown that naloxone reaches peak concentrations in brain by 15 min⁷⁰ and naltrexone by 30 min⁴⁶ after injection. In addition, maximal effects of naloxone on self-stimulation are typically obtained by 20–30 min after injection (Trujillo, unpublished observation). This was confirmed in the present study by administering naloxone immediately prior to the test session and following the time course of action for this drug.

Histological analysis

Upon completion of experiments, animals were given an overdose of chloral hydrate and perfused intracardially with saline followed by 10% formalin. Brains were removed, frozen, and sectioned at 40 μ m. Electrode placements were verified using the atlas of König and Klippel³⁸.

Data analysis

The number of lever presses during a particular time period in the experimental session was compared to the corresponding period in the preceding

control session, and is expressed as a percent of control. Paired *t*-test analysis assessed whether experimental effects were different from the control session. Latency to onset of suppression was determined by overlaying the cumulative response record from the experimental session on that of the control session and measuring the length of time from the beginning of the session to the point at which the experimental record deviated from the control (see Figs. 4 and 5). Data from animals that did not complete the series of experiments was used in the group data for those experiments that they did complete.

RESULTS

Of the 13 animals implanted with electrodes, two

were not used in experiments: one due to loss of the implant early in training, and the other due to unstable responding. Histological analysis revealed that all the remaining electrodes were located in the nucleus accumbens³⁸ (see Fig. 1). Animals averaged approximately 42 training sessions prior to experimental manipulations. Mean baseline response rate for nucleus accumbens self-stimulation at the beginning of experiments was 1342 ± 172 leverpresses/h., and mean baseline current intensity $198 \pm 19 \mu\text{A}$. Response rates were dependent on current intensity—reduction in current caused an intensity-related decrease in responding. This was observed during the rate-intensity current adjustments (Table II) and when current was reduced in experimental sessions ($77.9 \pm 5.3\%$ of control at 75% current intensity, $n = 7$, $P < 0.02$; $52.7 \pm 7.1\%$ of control at 50%

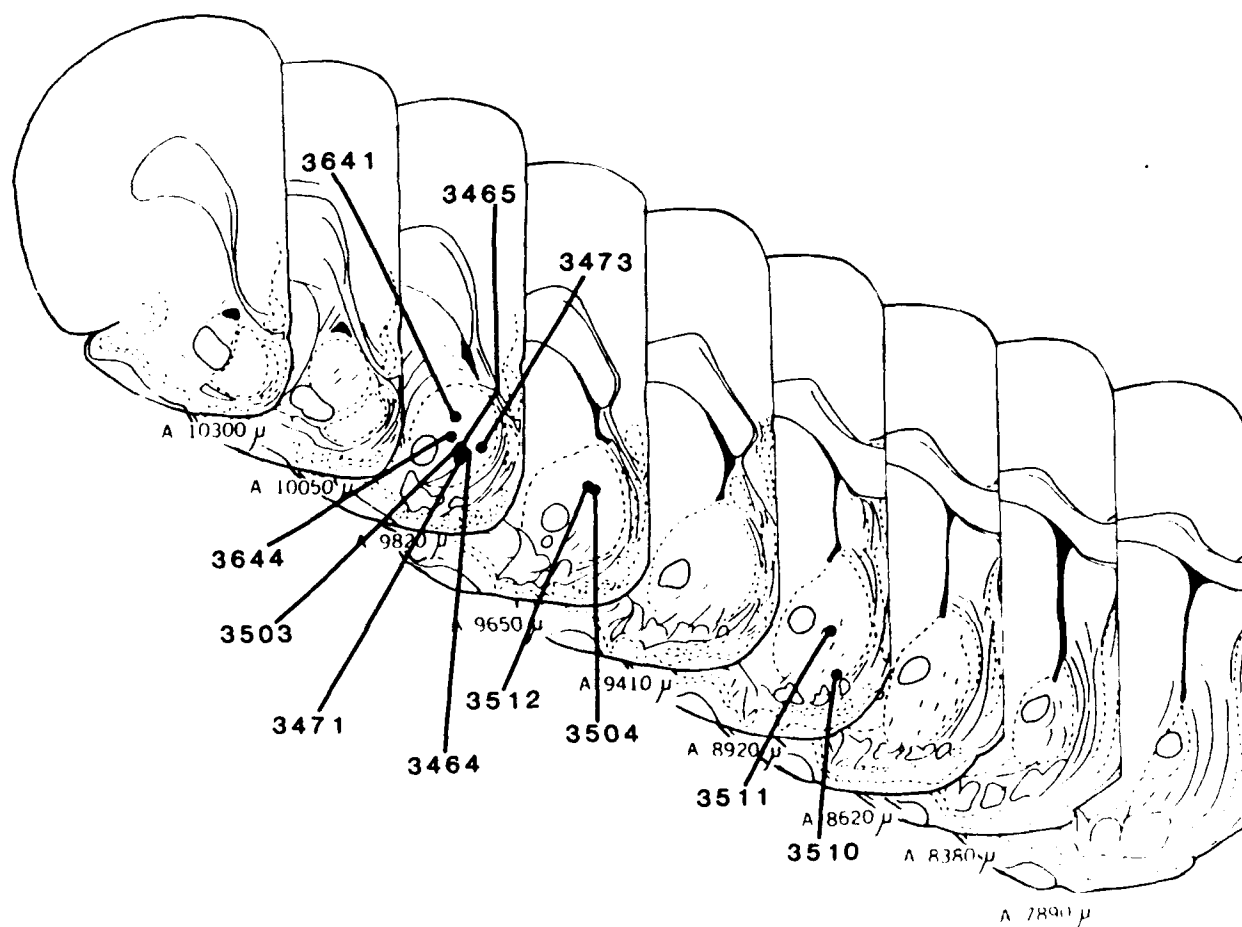


Fig. 1. Summary diagram of electrode placements. Electrode tips are indicated by filled circles on representative brain sections from the atlas of König and Klippel³⁸. All electrodes are in the nucleus accumbens, with 9 out of 11 in the anterior half of this nucleus. Numbers shown are identification numbers for animals used in these experiments.

current intensity, $n = 10$, $P < 0.01$). Naloxone (2.0 and 20 mg/kg s.c.) dose-dependently suppressed total response rates for nucleus accumbens self-

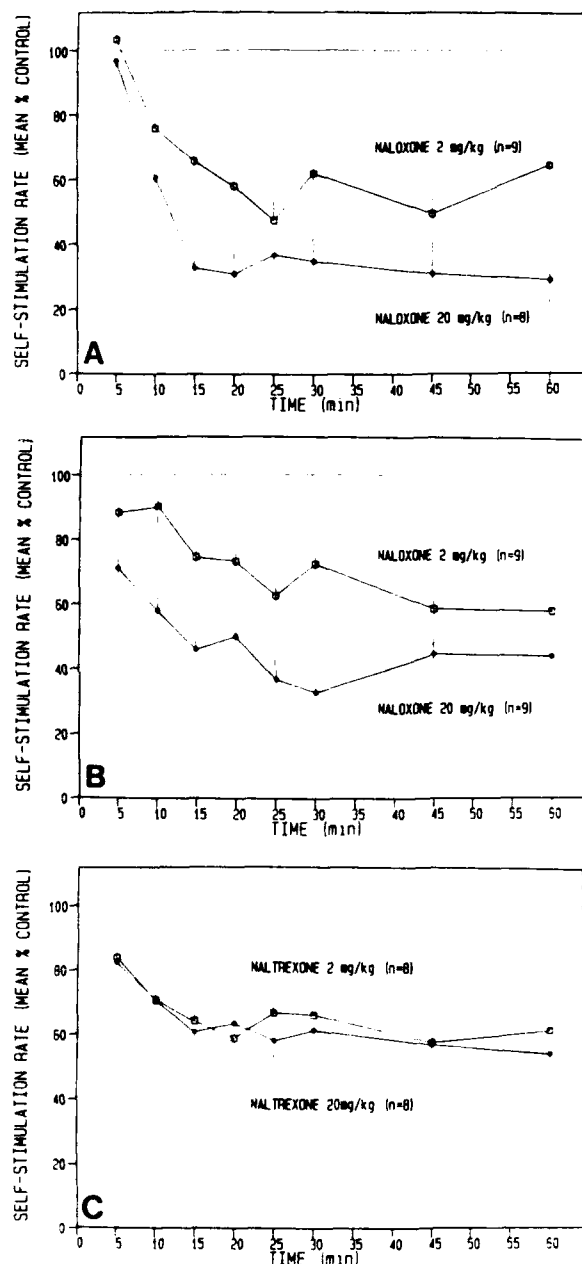


Fig. 2. Time course for the effects of naloxone and naltrexone on nucleus accumbens self-stimulation. (A) Naloxone (2.0 or 20 mg/kg s.c.) administered immediately prior to the test session. (B) Naloxone (2.0 or 20 mg/kg s.c.) administered 30 min prior to the test session. (C) Naltrexone (2.0 or 20 mg/kg s.c.) administered 30 min prior to the test session. Effects are expressed as percent control \pm S.E.M. of the corresponding time period of the preceding control session.

TABLE II

Response rate is dependent on current intensity

Data are from the rate-intensity sessions for the 11 animals in this experiment. Number of animals tested at each current intensity is identified in parentheses. Note that response rate decreases as current is decreased.

| Current intensity (μ A) | Mean number of responses 5 min |
|------------------------------|--------------------------------|
| 350 | 140 (10) |
| 300 | 127 (10) |
| 250 | 110 (11) |
| 200 | 99 (9) |
| 150 | 58 (8) |

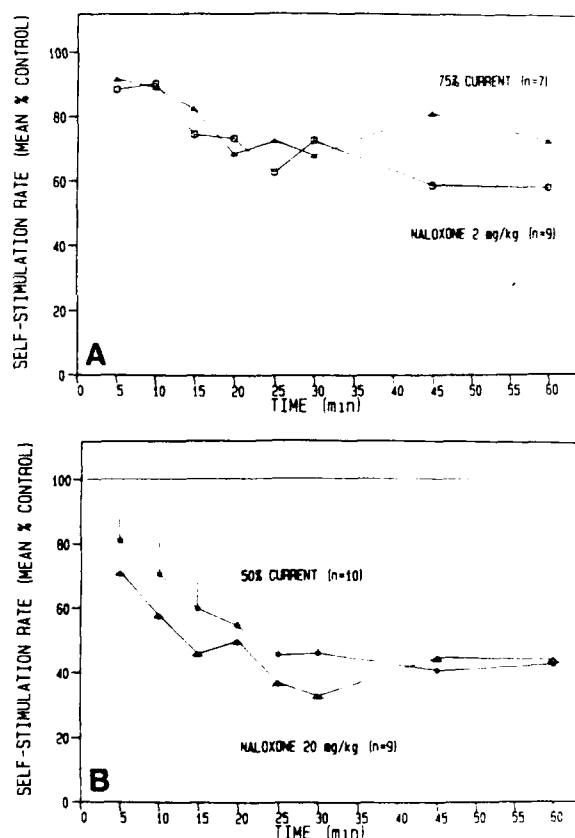


Fig. 3. Comparison between the response patterns produced by naloxone and reduction in current intensity on self-stimulation of the nucleus accumbens. (A) Naloxone (2.0 mg/kg) administered 30 min prior to the test session produced effects very similar to reducing the current to 75% of baseline current intensity. (B) Naloxone (20 mg/kg) administered 30 min prior to the test session produced effects very similar to reducing the current to 50% of baseline current intensity. Effects are expressed as percent control \pm S.E.M. of the corresponding time period in the preceding control session.

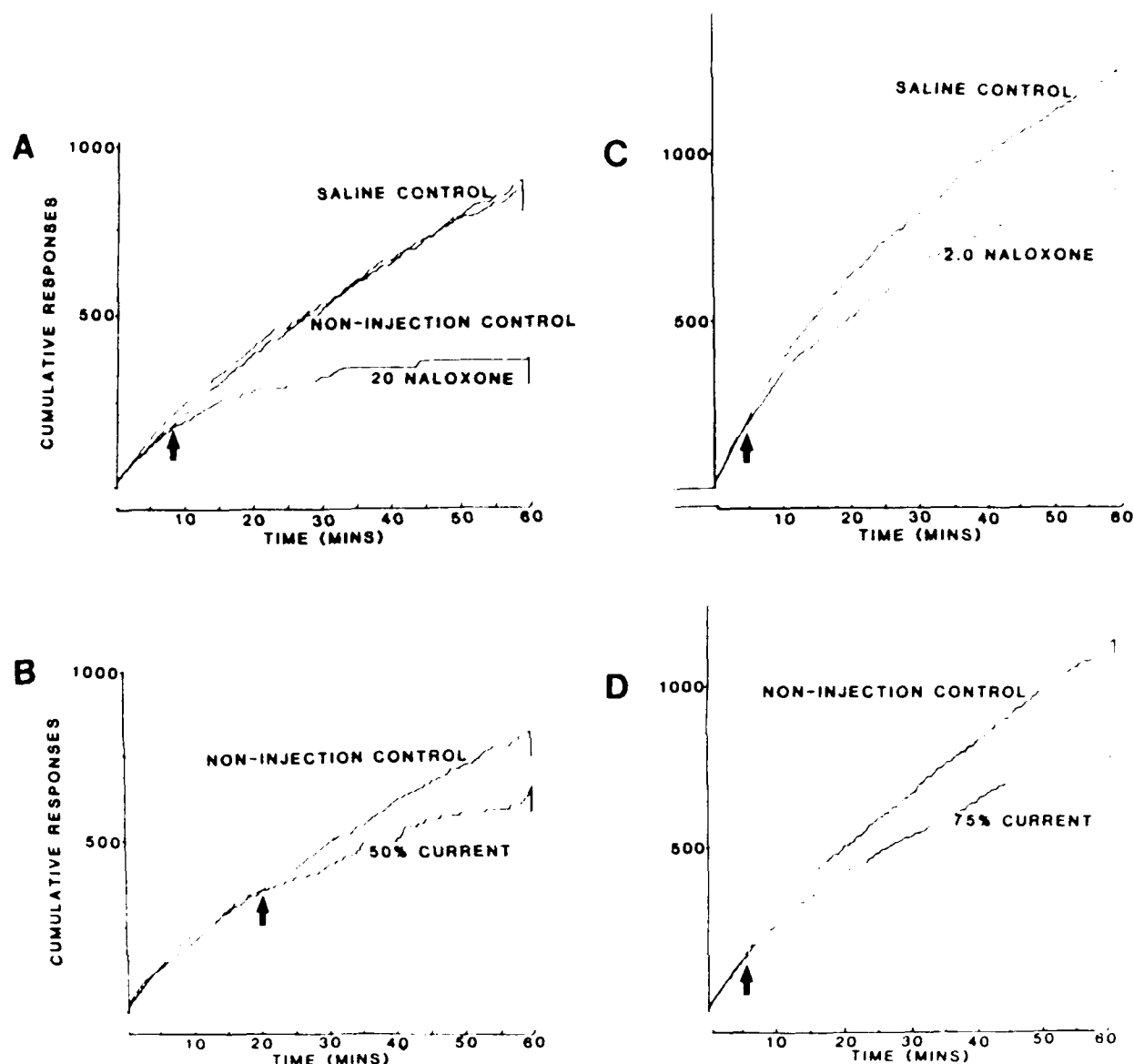


Fig. 4. Representative individual cumulative response records for nucleus accumbens self-stimulation. Arrows indicate the latency to onset of suppression (see Fig. 5). (A) Cumulative response record for animal no. 3503 self-stimulating under 20 mg/kg naloxone is compared to the immediately preceding saline control and non-injection control sessions. Note that the saline control record is virtually identical to the non-injection control record, while the naloxone record deviates downward, indicating suppression of responding. (B) Cumulative response record for animal no. 3503 self-stimulating at 50% current intensity is compared to the immediately preceding non-injection control session. (C) Cumulative response record for animal no. 3512 self-stimulating under 2 mg/kg naloxone compared to the immediately preceding saline control session. (D) Cumulative response record for animal no. 3512 self-stimulating at 75% current intensity is compared to the immediately preceding non-injection control session.

stimulation whether administered immediately prior ($64.4 \pm 5.5\%$, $n = 9$, $P < 0.02$; and $42.9 \pm 6.3\%$, $n = 8$, $P < 0.001$ relative to saline control, respectively) or 30 min prior ($69.9 \pm 3.6\%$, $n = 9$, $P < 0.001$; and $48.2 \pm 4.4\%$, $n = 9$, $P < 0.001$

relative to control, respectively) to the beginning of the test session, mimicking the effects of current reduction. Although administration of naloxone immediately prior to the session appeared to suppress self-stimulation slightly more than administra-

tion 30 min prior, no statistically significant differences in total response rates were observed between the two administration times. Naltrexone administered 30 min prior to the beginning of the test session also suppressed total response rates for self-stimulation. Effects at 2.0 mg/kg were quantitatively similar to naloxone at the same dose ($64.2 \pm 5.7\%$ of control), however they did not increase with the 20 mg/kg dose ($62.0 \pm 7.4\%$ of control).

The time course of drug effects is shown in Fig. 2. Naloxone administered immediately prior to the test session showed no effects on responding at the 5 min time point for either the 2.0 or 20 mg/kg dose. Suppression was observed at 10 min and increased during successive 5 min periods to a plateau of approximately 30% of control at 20 min for 20 mg/kg, and approximately 50% of control at 25 min for 2.0 mg/kg (Fig. 2A). Naloxone administered 30 min prior to the test session produced very similar effects, despite the fact that testing began 5–10 min *after* the time point at which maximal effects were seen following immediate administration. Although

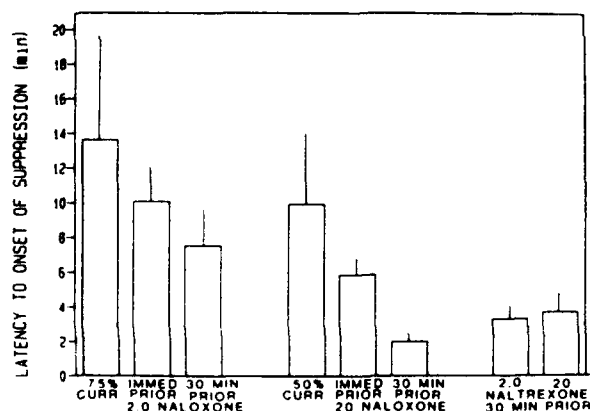


Fig. 5. Latency to onset of suppression for experimental manipulations of nucleus accumbens self-stimulation. Latency was quantified for each animal by overlaying the cumulative response record from an experimental session on that from the control session and determining the time point at which the experimental record deviated downward from the control record (see Fig. 4). Means were calculated for each experimental manipulation. Note that for each manipulation a non-zero latency to onset of suppression was observed. This illustrates that responding was normal during the first minutes of experimental sessions. Experimental manipulations are from the left to right: 75% current intensity; naloxone 2.0 mg/kg injected immediately prior and 30 min prior to the experimental session; 50% current intensity; naloxone 20 mg/kg injected immediately prior and 30 min prior to the experimental session; naltrexone 2.0 and 20 mg/kg injected 30 min prior to the experimental session.

a slight suppression was observed at 5 min (the first time point assessed) for 2.0 mg/kg, and a greater suppression at 5 min for 20 mg/kg, thereafter the pattern of suppression for the respective doses was very similar to that seen when the drug was administered immediately prior to the session (Fig. 2B). Initial high rates of response were observed, followed by suppression, with maximal effects again delayed 25–30 min after the beginning of the session (55–60 min after the injection). Similar effects were observed for naltrexone 2.0 and 20 mg/kg administered 30 min prior to the session (Fig. 2C). Although a small suppression was observed at 5 min, maximal effects were delayed 20–25 min after the beginning of the session (50–55 min after injection).

In order to determine if the response decrement pattern mimics that produced by a reduction in reinforcement, the effects of naloxone administered 30 min prior to the test session were compared to the effects produced by a reduction in current intensity. As can be seen (Fig. 3A), the response pattern produced by 2.0 mg/kg of naloxone is comparable to that seen at 75% current intensity and the pattern produced by 20 mg/kg is very similar to that seen at 50% current intensity (Fig. 3B). In addition, naltrexone at 2.0 and 20 mg/kg administered 30 min prior to the test session produced a pattern almost identical to the 75% current intensity session (compare Fig. 2C with Fig. 3A).

The extinction-like response decrement pattern is better illustrated by examining individual cumulative response records (Fig. 4). As can be seen, in each case shown (as well as for every case examined) there is a distinct latency from the beginning of the session to the onset of suppression for drug trials as well as for current reductions. These latencies are quantified and compared in Fig. 5. It is important to emphasize that for each manipulation (drug or current reduction) a non-zero latency to the onset of suppression was observed. This latency indicates that responding during the first minutes of the session was normal when compared to the preceding control session.

DISCUSSION

The present study provides evidence that opiate antagonists suppress self-stimulation behavior by

specific effects on reinforcement rather than by non-specific effects on performance. Naloxone and naltrexone produced extinction-like response decrement patterns in nucleus accumbens self-stimulation, with normal rates of response at the beginning of the session followed minutes later by suppression of responding. If these drugs had suppressed self-stimulation by non-specific effects on performance, suppression would have occurred throughout the session, without the initial normal response rates^{17-19,42}. It is important to emphasize that the increasing behavioral effects characteristic of the extinction pattern were observed despite the fact that testing began 30 min after the injection. This is several minutes after the time point at which maximal suppression of self-stimulation normally occurs (as demonstrated by naloxone administration immediately prior to the session in the present study), and when brain concentrations of naloxone⁷⁰ and naltrexone⁴⁶ were, in fact, declining. Maximal effects of the antagonists were not seen until 25–30 min after the start of the session, regardless of whether the drug was administered immediately prior or 30 min prior to the session. These results suggest that the response patterns produced by the opiate antagonists were not the result of increasing drug concentrations during the course of the session. This suggestion is highlighted by the fact that a similar response decrement pattern has been observed for naltrexone (10 mg/kg) administered 23 h prior to the experimental session⁷².

The effects of naloxone and naltrexone were very similar to, and in fact, overlapped and paralleled the effects of reducing current intensity. Reduction in current intensity can be considered a 'direct' decrease in the reinforcement value of the stimulation. The similarities in the response decrement patterns between the opiate antagonists and reductions in current intensity therefore support our suggestion that the antagonists are acting by decreasing reward, rather than interfering with performance. In an interesting parallel to the present results, extinction-like patterns have also been observed for naloxone suppression of stimulation-induced feeding⁷ as well as free feeding⁸³, suggesting that opiate antagonists specifically interfere with the reinforcing value of food.

Possible alternative explanations for extinction-

like effects have been termed 'response-produced performance deficit' by Ettenberg, Cinsavich and White¹⁶ and 'pseudoextinction' by Gallistel, Boytim, Gomita and Klebanoff²². These explanations require that the drug interact with a concomitant of responding, such as fatigue or seizure activity, to produce a progressive debilitation. Although response-produced performance deficit and pseudoextinction cannot be ruled out entirely in the present study, some comments can be made regarding these possibilities. In regards to a possible seizure-related debilitation, although seizures were occasionally seen in some animals in the present study, behavioral observation revealed no enhanced seizure activity in the presence of opiate antagonists – neither increased incidence of seizures in seizure-prone animals, nor induction of seizures in animals that had not previously experienced a seizure. In a review of the literature, Albertson, Joy and Stark¹ concluded that there are no consistent effects of opiate antagonists on electrically kindled seizures in rats. These observations, together with the extinction-like effects of naloxone observed on free feeding⁸³ suggest that the extinction pattern does not result from a seizure-enhancing mechanism. Likewise, response-produced performance deficit does not appear to be the mechanism by which opiate antagonists suppress self-stimulation. According to Ettenberg et al.¹⁶ such a deficit can result from peripheral actions of a drug that cause the animal to tire after a relatively small amount of responding. However, quaternary derivatives of naloxone and naltrexone have been found to have no effects on self-stimulation, suggesting that opiate antagonists affect this behavior by acting in the brain rather than the periphery^{4,60}. In addition, naloxone has been observed to suppress lever-pressing and nose-poking for self-stimulation equally. Since lever-pressing requires more motor output than nose-poking, fatigue would be expected to affect the former more strongly⁴. Further, the relative lack of potency of opiate antagonists on locomotor activity as compared to self-stimulation⁸⁰ adds support to the suggestion that fatigue is not the mechanism by which these drugs suppress self-stimulation.

Another alternative possibility for the effects of opiate antagonists on self-stimulation is that these drugs, rather than decreasing reinforcement, en-

hance the aversive properties of the stimulation^{21,32}. The hypothesized role for endogenous opioids, therefore, would be to suppress aversion. This is supported firstly, by the fact that opioids have potent analgesic effects, and secondly, by demonstrations that naloxone increases responding⁶⁸ and decreases threshold⁵⁶ for escape from aversive brain stimulation. However, recent evidence suggests that opiate antagonists suppress self-stimulation by decreasing reward rather than by increasing aversion³⁷. Although other possible alternatives can probably be proposed to explain the present effects, the most parsimonious explanation for the response pattern produced by opiate antagonists is a reduction in the reinforcing value of the stimulation.

Interestingly, while naloxone produced dose-dependent suppression of self-stimulation, the effects of naltrexone at 20 mg/kg were not greater than at 2.0 mg/kg. Similar results have previously been observed for the effects of these drugs on self-stimulation of the medial forebrain bundle⁵⁸ and on fluid intake in deprived animals³⁰. This is a curious observation since naltrexone has a higher affinity than naloxone for μ -, κ - and δ -receptors in *in vitro* experiments⁴⁵. One possible explanation is that the 2 mg/kg dose of either naloxone or naltrexone is enough to prevent the involvement of these 3 receptor types in reinforcement. The additional effects of naloxone at the higher doses might then be due to action at a different receptor, either opiate or non-opiate, that naltrexone does not affect. As such, low doses of naloxone may specifically block the reinforcing effects of self-stimulation, while higher doses may, in addition, affect performance. Consistent with this suggestion, studies have found that locomotor activity is suppressed by naloxone at doses of 10 mg/kg or greater, but not smaller doses^{2,13,27,29}.

As discussed above, electrode placement and length of test session appear to be particularly important factors in observing suppression of self-stimulation with opiate antagonists, although other factors such as schedule of reinforcement and stimulation parameters may also contribute^{21,80}. It is not difficult to reason why electrode placement might be important in observing the effects of opiate antagonists on self-stimulation behavior, particularly since a second class of neurotransmitters, the catechol-

amines, appear to be highly involved in this behavior⁶⁷. The reinforcing effects of stimulation at different sites involves different neurotransmitters – at some sites endogenous opioids are important, at some sites catecholamines are important, and at other sites both are involved. The observation that different drug effects occur at different electrode sites is strong evidence that the suppression seen is not the result of a performance deficit⁴². If non-specific debilitation were responsible for the effects of opiate antagonists, then the behavior should be suppressed regardless of where the electrode is located. Although not unanimous²⁰, several studies have reported differential effects of opiate antagonists at different implant sites^{21,23,40,41,44,58,68}. Since the nucleus accumbens appears to be a site at which both catecholamines and endogenous opioids are involved in the reinforcement⁷¹ it is not surprising that self-stimulation of this nucleus is not completely abolished by opiate antagonists, but merely attenuated – elimination of the endogenous opioid component of self-stimulation with opiate antagonists still allows for expression of the catecholamine component.

Although it is not immediately obvious as to why long test sessions might be important for observing the effects of naloxone and naltrexone on this behavior, the present study demonstrating an initial period of normal responding prior to suppression of self-stimulation offers an explanation. Since there is a latency on the order of minutes prior to the onset of suppression, it is not surprising to find that studies using short sessions typically do not observe effects of these drugs on self-stimulation; particularly studies using sessions as short as two⁶, three⁶⁵, or 10 min^{43,47,48}. The question that then arises is why do opiate antagonists require several minutes to act in self-stimulation, when extinction normally occurs very rapidly in this paradigm after turning off the current⁴⁹? The answer lies in the perceptual abilities of the animal to discriminate the change in reward value that has taken place as a result of the drug treatment. As noted above, self-stimulation in the presence of opiate antagonists is not eliminated, but merely attenuated. It has been suggested that the rate of extinction depends on the ease with which the animal can discriminate the change in the reinforcement contingency³⁴. The slow extinction-like pattern

therefore occurs because the reduction in reinforcement caused by opiate antagonists is a relatively subtle change compared to complete elimination of reinforcement. This is supported by the observation in the present study that the pattern of responding in the presence of opiate antagonists is very similar, if not identical, to that seen with graded reductions in current intensity.

Although the present study provides evidence that opiate antagonists interfere with the reinforcing properties of self-stimulation, they do not necessarily indicate a role for endogenous opioids in this behavior. Sawynok, Pinsky and LaBella⁵⁷ have emphasized that the action of naloxone on a particular process is a necessary although not sufficient criterion for demonstrating involvement of endogenous opioids, since this drug may have effects other than opiate receptor blockade. Nevertheless, several lines of evidence suggest that endogenous opioids are indeed involved in brain-stimulation reward. First, the low doses required to suppress self-stimulation offers evidence that the actions of naloxone are due to blockade of endogenous opioids. Consistently in our laboratory, we have observed that doses as low as 0.2 mg/kg of naloxone will suppress nucleus accumbens self-stimulation, with an ED₅₀ of between 0.2 and 2.0 mg/kg^{4,71}. This is in the dose range that is typically used to block the actions of endogenous opioids²⁸. Second, the observation in the present experiments, as well as in previous studies^{4,36,58,59}, that both naloxone and naltrexone suppress this behavior suggests that the action is the result of opiate receptor blockade and not a side effect of a particular antagonist. Third, the recent demonstration that brain opioid peptide levels are altered by brain-stimulation reward, but not non-contingent stimulation, and that the changes correlate well with the performance of the animal in self-stimulation⁶⁶, offers further evidence that endogenous opioids are indeed involved in the reinforcing effects of brain stimulation.

While the present paper was under revision, West and Wise⁸¹ reported the lack of an extinction-like response decrement pattern for naltrexone in self-stimulation. There are, however, some important differences between the present experiments and those of West and Wise. First, in the present experiments we used animals with electrodes im-

planted into the nucleus accumbens, while West and Wise examined animals with electrodes in the lateral hypothalamus. As noted above, as well as in the West and Wise paper, the nucleus accumbens is a brain region in which self-stimulation is particularly sensitive to opiate antagonists, while the lateral hypothalamus is relatively refractory to the effects of these drugs. Second, animals in our study were placed in the test chamber 30 min after injection, and examined in 60-min sessions, without priming, but with stimulation available during the entire session. Animals in the West and Wise study were examined in 2-min test sessions, every 20 min, remaining in the test chambers during the time-out periods, as well as during the 45 min following the injection, and primed with non-contingent stimulations if they did not respond. These differences, or other more subtle differences between the studies may have contributed to the differing results. It is therefore important to examine the effects of opiate antagonists on self-stimulation behavior further, in order to determine if the extinction-like response pattern is specific to a particular set of experimental conditions, or if this effect can be seen under a variety of conditions. It should be noted that other studies have reported suggestions of extinction-like response decrement patterns from opiate antagonists in self-stimulation experiments. Stapleton and co-workers⁶⁵ and Collier and Routtenberg⁹, although not systematically studying response patterns, observed that naloxone was less effective during the initial phase of responding than later in the session. These anecdotal reports suggest that the extinction-like response decrement pattern for opiate antagonists may indeed generalize to a variety of self-stimulation sites and experimental conditions.

In summary, the present study, by demonstrating that naloxone and naltrexone produce extinction-like response decrement patterns on self-stimulation behavior, provides evidence that opiate antagonists suppress this behavior by specific effects on reinforcement, rather than by non-specific effects on performance. These results support the suggestion that endogenous opioids are responsible, at least in part, for the reinforcing effects of brain stimulation. In addition to brain-stimulation reward, the endogenous opioids may play an important role in the reinforcing effects of feeding, drinking, and drugs of

abuse (see for reviews refs. 10, 11, 54). These peptides therefore appear to be mediators of a variety of rewarding stimuli, and as a result may be

important neurotransmitters in normal goal-directed behavior, as well as in abnormal reward conditions such as depression, schizophrenia and drug abuse.

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Naloxone Suppression of Self-Stimulation Is Independent of Response Difficulty¹

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TRUJILLO, K. A., J. D. BELLUZZI AND L. STEIN. *Naloxone suppression of self-stimulation is independent of response difficulty.* PHARMACOL BIOCHEM BEHAV 33(1): 147-155, 1989.—The action of the opiate antagonist naloxone on relatively easy (nose-poke) and relatively difficult (lever-press) self-stimulation behaviors was compared, in order to determine if opiate antagonists suppress self-stimulation by interfering with the ability of the animal to respond, or by reducing the reinforcement value of the stimulation. Naloxone (0.2, 2.0 and 20 mg/kg) significantly suppressed both nose-poking and lever-pressing self-stimulation rates, and the degree of suppression was virtually identical for both tasks at all doses examined. If naloxone had interfered with the ability of the animal to respond, then lever-pressing—which requires more motor output than nose-poking—should have been more suppressed than nose-poking. The results suggest that opiate antagonists do not interfere with the ability of the animal to respond, and are therefore consistent with the hypothesis that these drugs reduce the reinforcement value of the stimulation.

Naloxone Self-stimulation Lever-press Nose-poke Reinforcement Endorphins Response difficulty

EVIDENCE from a variety of studies suggests that endogenous opioids are important in reinforcement function. This is supported by the observation that self-stimulation behavior is suppressed by the opiate antagonists naloxone and naltrexone (2, 4, 22, 31-33, 35, 36, 39, 40, 44, 46, 47). While failures to find suppression of self-stimulation by opiate antagonists have been reported (29, 38, 42), the specific methodology used plays a critical role: opiate antagonists do indeed suppress self-stimulation if sensitive methods are used (31, 35, 40, 46). Interpretation of the suppression of self-stimulation by these drugs remains a matter of controversy. While some investigators suggest that opiate antagonists suppress self-stimulation principally by blocking the reinforcing effects of stimulation-released endogenous opioids (2, 4, 22, 32, 36, 40), others believe that the effects of these drugs result from motor incapacitation or other nonspecific performance deficits (14, 29, 38). The latter interpretation is apparently supported by studies that demonstrate suppressive effects of opiate antagonists on locomotor behavior (1, 8, 20, 21). However, the effects of these compounds on locomotion are relatively subtle and occur at doses higher than those necessary to suppress self-stimulation [see (46)]. The present study is a further attempt to determine if opiate antagonists suppress self-stimulation by interfering with reward or motor performance.

Distinctions between reinforcement and performance deficits can be made by comparing drug effects on self-stimulation responses that differ in difficulty. Drugs that cause motor debilitation should produce greater impairment of a difficult response than of a simple one. On the other hand, drugs that primarily interfere with reinforcement function should suppress different

responses equally (26). Nose-poking is a natural exploratory behavior for the rat—these animals typically explore an environment by actively poking their noses into holes and corners. In contrast, lever-pressing is a less natural and more complex act for this animal. Gerhardt and Liebman (16) have demonstrated in self-stimulation experiments that lever-pressing is more susceptible than nose-poking to suppression by drugs that affect the motor capacity of the animal, while the two responses are suppressed equally by compounds thought to act specifically on reinforcement function. Thus, while the hypnotic pentobarbital and the muscle relaxant methocarbamol suppressed lever-pressing to a greater extent than nose-poking, the dopamine antagonist haloperidol suppressed both tasks equally.

In the present study, the effects of naloxone on self-stimulation of the nucleus accumbens was determined in the rat, using nose-poking and lever-pressing as response measures. The nucleus accumbens contains high concentrations of opioid peptides and opiate receptors, and self-stimulation of this nucleus is sensitive to suppression by opiate antagonists (3, 22, 35, 39, 40). Furthermore, several studies implicate the nucleus accumbens in the mediation of the reinforcing effects of opioids (19, 28, 34, 41, 43). If, at such an opioid-dependent site as the nucleus accumbens, response difficulty was the major determinant of naloxone's suppressant action on self-stimulation, then the motor impairment hypothesis would be supported. In contrast, a lack of involvement of response difficulty in the effects of naloxone would be consistent with the hypothesis that endogenous opioids play an important role in the reinforcing properties of self-stimulation.

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METHOD

Animals

Experimentally-naïve male albino Sprague-Dawley rats (Charles River) were used. The animals weighed 305 to 335 g at the time of surgery, and were individually housed on a 12-hour light/dark cycle with food and water available at all times.

Surgery

Rats were anesthetized with sodium pentobarbital (50 mg/kg IP) and stereotactically implanted with bipolar electrodes (Plastic Products MS 303-8) aimed at the nucleus accumbens (coordinates: skull level with horizontal: A-P = -2.0 mm from bregma; Lat. 1.2 mm from midline; D-V = -6.0 from the brain surface). Electrodes were attached to the skull using stainless steel screws and dental cement.

Apparatus

Twelve chambers (28 × 25 × 30 cm high) each containing a lever (3.8 × 1.3 × 1.5 cm) mounted on the rear wall 4 cm above the grid floor were used for lever-press experiments. A light located above the lever remained on when the stimulation was available. A second (inactive) lever was located on the door of the chamber; responses at this lever were counted, but produced no stimulation. Chambers were constructed of Plexiglas with black rear and side walls, and clear door and ceiling. The same chambers were used for nose-poke experiments except that the door was replaced with one containing an 8 × 14 cm stainless steel panel. The panel had two 1.5-cm holes located side by side (6.5 cm apart; 4 cm from the grid floor), each of which contained a photocell apparatus. A light located above the active hole remained on when the stimulation was available. Nose-pokes through the inactive hole were counted but produced no stimulation. A white Plexiglas wall blocked access to the rear lever during nose-poke experiments. Self-stimulation chambers were individually housed in sound-insulated compartments with white noise. A single lever-press (10 g force required) or nose-poke (no force required; animal needed only to break a light beam with its nose) delivered a 150 msec train of electrical brain stimulation consisting of monophasic rectangular pulses of 0.2 msec duration presented at 100 pulses per second through an isolation transformer. Electrical connection through a commutator allowed the rat free movement in the chamber at all times. Responses were automatically counted and recorded at five-minute intervals by a computer interfaced with the chambers via a BRS-LVE Interact system. In addition, cumulative recorders continuously monitored responding throughout the session.

Experiment 1 Procedure

Animals were trained to nose-poke for self-stimulation at 350 μ A current intensity in 60-minute sessions, five days per week. After stable response rates were achieved, a descending rate-intensity function was determined for each rat to identify the lowest current that would maintain stable responding. This was achieved in a single self-stimulation session as follows: rats began responding in self-stimulation at 350 μ A current intensity as normal. Current intensity was readjusted downward by 25 to 50 μ A every five minutes, until responding became disrupted or intermittent. At this point, current was adjusted up and down around this intensity to establish the lowest value that would maintain stable responding. This current intensity was identified for each animal as the "baseline" current and was maintained at the new value for the remaining nose-poke sessions. Drug exper-

iments began after response rates restabilized at the new "baseline" current intensities. Since drug effects are more pronounced at low currents than at maximal ones, use of these low baselines provides a more sensitive assessment of the reinforcement mechanisms underlying the self-stimulation behavior than use of higher current intensities (44).

During drug experiments, animals were tested seven days per week, with naloxone doses (0.2, 2.0 and 20 mg/kg) administered in a random order, and with at least 3 days between drug injections. A saline injection on the day prior to each drug injection served as the control for that drug test. If response rate changed by more than 10% during the saline session the drug test for that animal was postponed another 3 days. Naloxone HCl was dissolved in sterile saline and administered subcutaneously (SC) in a volume of 1 ml/kg immediately prior to the experimental session. After receiving all naloxone doses in nose-poke tests, animals were switched to lever-press. Current intensities were readjusted over the following days in order to equate lever-press response rates with those observed during nose-poking. After responding restabilized at the new baseline current intensity, animals again received all naloxone doses as described above.

Experiment 2 Procedure

Animals were allowed to self-stimulate at 350 μ A current intensity in 60-minute sessions, five days per week. The first seven days, animals were exposed on alternate days to nose-poke and lever-press, counterbalanced for order of exposure across rats; i.e., some rats received exposure to nose-poke on the first day while others experienced lever-press during this session, followed by the alternate task the following day. Therefore, each animal experienced nose-poke and lever-press conditions for at least three days each during this period. Following this period of acquisition, half of the animals were assigned to nose-poke and half to lever-press for further training. After stable response rates were obtained at 350 μ A current intensity, a descending rate-intensity function, as described above, was determined for each rat to identify the lowest current that would maintain stable responding. Current intensity remained at the new value for the remainder of the animal's history. Drug experiments began after response rates restabilized at the new "baseline" current intensities. During the course of drug experiments animals were allowed to self-stimulate five days per week. Days one, two, and five, animals received no treatment; day three served as saline control session; injections of 0.2, 2.0 or 20 mg/kg naloxone HCl occurred on day four. Doses were presented in a random order, and at least seven days separated drug injections. If response rate changed by more than 10% during the saline session, no experimental manipulation was performed that week. Naloxone was dissolved in sterile saline and administered subcutaneously (SC) in a volume of 1 ml/kg immediately prior to the experimental session. After all doses were tested in the first task, animals were switched to the alternate task (i.e., if they were lever-pressing, they were switched to nose-poking, and vice versa), maintained at the same current intensity, and all doses were again administered as described above.

Histological Analysis

Upon completion of experiments, animals were given an overdose of chloral hydrate and perfused intracardially with saline followed by 10% formalin. Brains were removed, frozen, and sectioned at 40 μ . Electrode placements were verified using the atlas of König and Klippel (23).

Data Analysis

The number of lever presses during the final 45 minutes of a

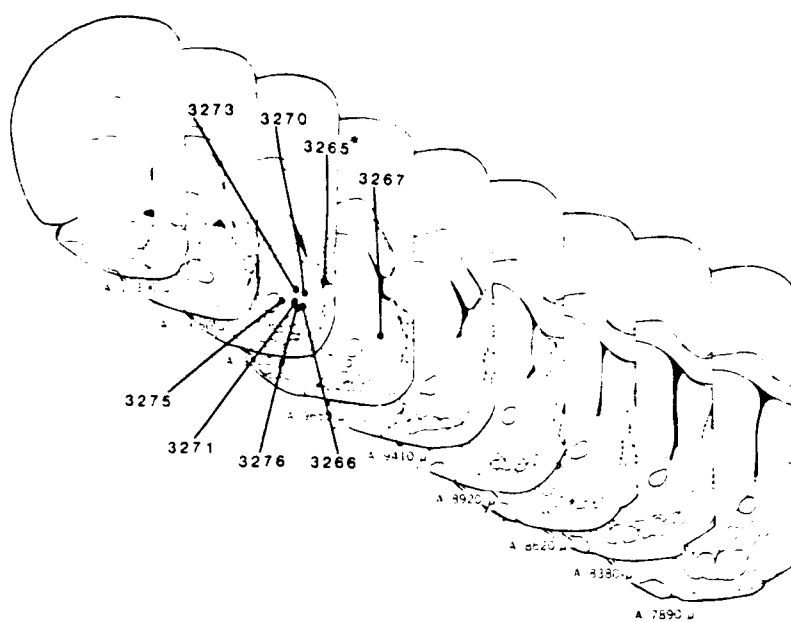


FIG. 1. Summary of diagram of electrode placements in Experiment 1. Electrode tips are indicated by filled circles on representative sections from the atlas of König and Klippel (23). Animal identification numbers are shown in bold. Seven out of eight electrodes are in the nucleus accumbens. The remaining electrode (indicated by *) is slightly medial to the accumbens.

drug session was compared to the final 45 minutes of the preceding saline control session and are expressed as mean percent of control. Paired *t*-test analysis assessed whether experimental effects were different from control, or whether nose-poke was different from lever-press.

RESULTS

Experiment 1

Of the 12 animals implanted with electrodes, eight completed drug testing on both nose-poke and lever-press tasks. Of the four that did not finish, two died of illness and two were lost due to electrode problems. Histological analysis revealed that seven out of eight of the electrode tips were located in the nucleus accumbens (Fig. 1). The electrode tip for the eighth animal was located just medial to the accumbens; since self-stimulation behavior and drug effects for this animal were similar to the nucleus accumbens rats, the data were included in the analysis. Response rates were dependent on current intensity—reduction in current resulted in an intensity-related reduction in responding, as observed in the rate-intensity session (Table 1). The difference in response difficulty between nose-poke and lever-press is indicated by the increase in current intensity necessary after the switch in task to attain the same level of responding on lever-press as seen on nose-poke (Table 2; although the current intensity was substantially higher on lever-press, the difference was not statistically significant). Naloxone dose-dependently suppressed both nose-poking and lever-pressing for self-stimulation. In addition, the effects of this drug were nearly identical for both tasks at all three doses tested (Fig. 2).

Experiment 2

Seventeen of 25 animals implanted with an electrode were used

in the experiments. Of the eight that did not finish, seven lost their electrodes and one had repeated seizures during self-stimulation. Histological analysis revealed that 16 out of 17 electrode tips were located in the nucleus accumbens (Fig. 3). The seventeenth electrode was located adjacent to the accumbens in the anterior olfactory nucleus. Since the self-stimulation behavior and drug responses of this animal were no different than the remaining 16 animals, the data were included in the analysis.

Response rates during acquisition for nose-poking were greater than those for lever-pressing (Fig. 4), supporting the suggestion that lever-pressing is more difficult for the rat than nose poking. Rate-intensity data revealed that response rates were dependent on current intensity—reduction in current resulted in an intensity-related reduction in responding (Table 3). Note that at each current intensity except the highest, response rates for nose-poke were greater than for lever-press.

Nine animals received their first drug treatment on nose-poke.

TABLE 1
RATE-INTENSITY DATA FOR ANIMALS IN EXPERIMENT 1

| Current Intensity (μ A) | Mean Number of Responses 5 min |
|------------------------------|--------------------------------|
| 350 | 121 (8) |
| 300 | 100 (8) |
| 250 | 88 (8) |
| 200 | 89 (8) |
| 150 | 61 (8) |
| 100 | 62 (5) |

Data are from the rate-intensity sessions for the eight animals in Experiment 1. Number of rats tested at each intensity is identified in parentheses. Note that response rate decreases as current is decreased.

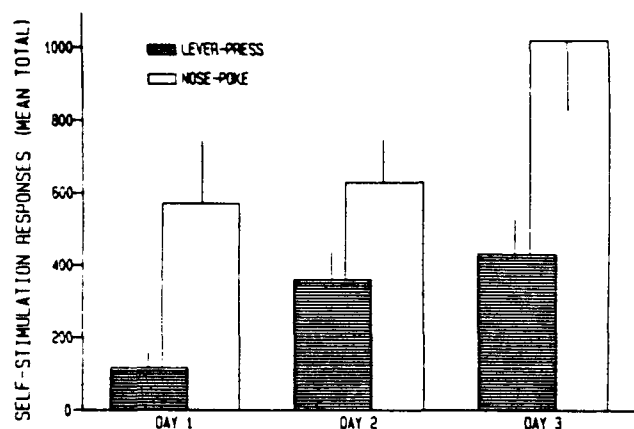


FIG. 4. Initial three days of acquisition for nose-poke and lever-press: Experiment 2. Each animal received exposure to nose-poke and lever-press on alternate days during the first days of self-stimulation training. Data are expressed as the mean total number of responses for all animals ($n = 24$) for the first, second and third exposure to each task (Day 1, Day 2 and Day 3, respectively). Note that for each exposure, the responding in nose-poke is greater than in lever-press (Day 1: lever-press = 117 ± 66 , nose-poke = 575 ± 174 , $p < 0.005$; Day 2: lever-press = 360 ± 127 , nose-poke = 632 ± 171 , $p < 0.05$; Day 3: lever-press = 432 ± 95 , nose-poke = 1019 ± 190 , $p < 0.01$).

nose-poke group were substantially higher than before the switch (Table 5; Fig. 7). On the other hand, in the animals that were switched from nose-poke to lever-press, the stabilized response rates were only slightly higher than before the switch (Table 5; Fig. 7).

The effects of naloxone were also dependent on the direction of the switch. In the animals that were switched from nose-poke to lever-press, the effects of naloxone were identical for both tasks at all doses (Fig. 8). In contrast, in the animals that were switched from lever-press to nose-poke, naloxone was less effective (although not significantly) on nose-poke at all doses (Fig. 9).

DISCUSSION

The present experiments compared the effects of naloxone on

TABLE 3
RATE-INTENSITY DATA FOR ANIMALS IN EXPERIMENT 2

| Current Intensity (μA) | Mean Number of Responses 5 min | |
|-------------------------------|--------------------------------|---------------------|
| | Nose-Poke Animals | Lever-Press Animals |
| 350 | 134 (9) | 135 (8) |
| 300 | 130 (9) | 119 (8) |
| 250 | 116 (9) | 108 (8) |
| 200 | 114 (9) | 80 (8) |
| 150 | 92 (8) | 35 (7) |
| 100 | 56 (8) | —* |

Data are from the rate-intensity sessions for the 17 animals in Experiment 2. Number of animals tested at each intensity is identified in parentheses. *Only two out of eight lever-press animals were responding at this current intensity making for an invalid comparison (one had 27 responses and the other 93 responses for the 5-minute period). Note that response-rate decreases as current is decreased, and that lever-press animals respond less than nose-poke animals for each current intensity except the highest.

TABLE 4

BASELINE CURRENT INTENSITY AND SALINE CONTROL RESPONSE RATE FOR NOSE-POKE AND LEVER-PRESS ANIMALS PRIOR TO THE SWITCH IN TASKS: EXPERIMENT 2

| | Baseline Current Intensity (μA) | Response Rate* |
|-------------|--|----------------|
| Lever-press | 181 ± 9.1 | 808 ± 62 |
| Nose-poke | 172 ± 11.4 | 792 ± 74 |

*Response rate is expressed as the mean number of responses \pm standard error for the final 45 minutes of saline control sessions ($n = 8$ animals, 3 determinations for each animal for lever-press; $n = 9$ animals, 3 determinations for each animal for nose-poke). There are no statistical differences between groups on either measure as determined by an independent t -test analysis.

two self-stimulation responses that differ in difficulty, lever-press and nose-poke, in order to determine if opiate antagonists suppress self-stimulation by interfering with the ability of the animal to respond. If opiate antagonists suppressed self-stimulation by interfering with performance, naloxone would have affected the more difficult response (lever-pressing) more strongly than the simpler response (nose-poking) (16,26). Our results show that naloxone affects nose-poke and lever-press similarly, suggesting that opiate antagonists do not interfere with the ability of the animal to perform the self-stimulation response.

Several observations support the suggestion that lever-pressing is indeed a more difficult task than nose-poking. First, nose-poking is a species-specific behavior requiring little movement and no force, while lever-pressing is a less natural response requiring more complex motor output and 10 grams of force. Second, increased current intensity was necessary to maintain response rates after switching the animals from nose-poke to lever-press in Experiment 1. Third, consistently higher response rates were

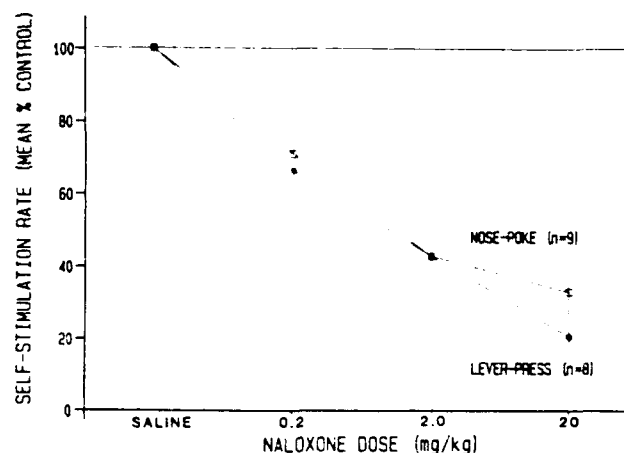


FIG. 5. Effects of naloxone on nose-poking and lever-pressing for nucleus accumbens self-stimulation: Experiment 2. Data points represent mean percent control \pm standard error. Naloxone significantly suppressed self-stimulation for each task at all doses examined [Nose-poke ($n = 9$): 0.2 mg/kg, $71.3 \pm 10.1\%$ of saline control, $p < 0.01$; 2.0 mg/kg, $42.9 \pm 11.9\%$, $p < 0.001$; 20 mg/kg, $32.7 \pm 5.9\%$, $p < 0.001$; Lever-press ($n = 8$): 0.2 mg/kg, $66.7 \pm 7.1\%$, $p < 0.01$; 2.0 mg/kg, $42.4 \pm 7.1\%$, $p < 0.01$; 20 mg/kg, $20.8 \pm 5.2\%$, $p < 0.01$]. There were no significant differences between the two tasks at any dose.

TABLE 5
EFFECTS OF SWITCH IN TASK ON CONTROL RESPONSE RATES IN
SELF-STIMULATION

| Response Rate | | % Change in Response Rate |
|---------------|-------------|------------------------------|
| Lever-press | Nose-poke | |
| 766 ± 70 | 1152 ± 114 | 144 ± 5.4* |
| Nose-poke | Lever-press | |
| 864 ± 104 | 1016 ± 153 | 113 ± 6.9* |

Response rates for the final 45 minutes of all saline control sessions (mean ± standard error), and % change resulting from the switch in tasks are shown for animals examined on both nose-poke and lever-press. Arrows represent direction of the switch in tasks.

*Significant difference in response rates on the two tasks, $n=7$, $p<0.001$; †significant difference in rates, $n=6$, $p<0.05$, as determined by paired t -test analysis.

obtained on the nose-poke task during response acquisition in Experiment 2. Similar differences in acquisition between these tasks were also noted by Gerhardt and Liebman (16). Fourth, in rate-intensity sessions, nose-poke rates were typically higher for a given current intensity than lever-press rates. Finally, in Experiment 2, rats switched from nose-poke to lever-press initially decreased responding, whereas rats switched from lever-press to nose-poke increased responding.

In Experiment 1 careful attention was paid to equalizing the response rates obtained on nose-poke and lever-press trials; therefore, drug trials were performed on rats responding for different current intensities on the two tasks. Nevertheless, naloxone suppressed nose-poke and lever-press responses equally.

In Experiment 2, in the initial between-group analysis of nose-poke and lever-press, baseline current intensity and response rate were unexpectedly equivalent. Although there was a slight tendency in the nose-poke group toward a lower baseline current intensity, this was not significant. The fact that baseline current intensity and response rate did not differ between the two groups of animals aids the interpretability of the comparison: the impor-

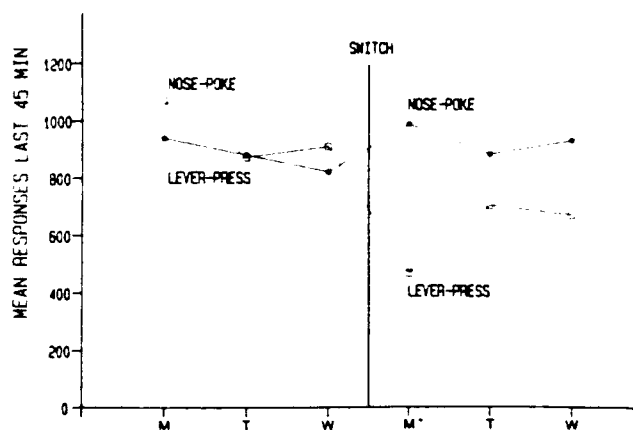


FIG. 6. Immediate effects of switch in task on response rates for self-stimulation. Experiment 2. The mean number of responses during the final 45 minutes of the session on Monday (M), Tuesday (T) and Wednesday (W) prior to the switch, and M, T and W after the switch in task are shown. Note that responding decreased for the group switched from nose-poke to lever-press, while responding slightly increased for the group switched from lever-press to nose-poke.

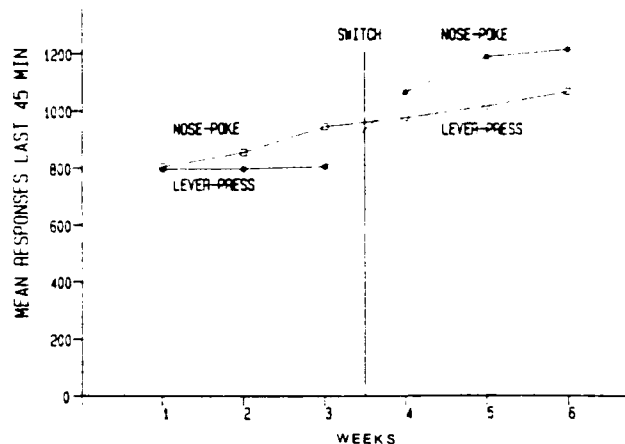


FIG. 7. Effects of switch in task on response rates for self-stimulation. The mean number of responses during the final 45 minutes of the session for each consecutive saline control day is shown before the switch and after the switch in task. Data shows the large increase in control rate for the animals switched from lever-press to nose-poke, as well as the change in rate over time that is typically observed in nucleus accumbens self-stimulation.

tance of having these factors equivalent when comparing different tasks in self-stimulation has been previously stressed (13, 16, 26). The actual differences between the tasks may have been masked by the variability in the two groups of rats. This is supported by the differences in response rate observed when the animals were switched to the opposite task.

As was seen in Experiment 1, the effects of naloxone in the between-group analysis in Experiment 2 were very similar for animals nose-poking and lever-pressing. Interestingly, in this comparison, while the two tasks were suppressed equally by naloxone at the lower doses (0.2 and 2.0 mg/kg), at the highest dose (20 mg/kg) lever-pressing was slightly more suppressed than

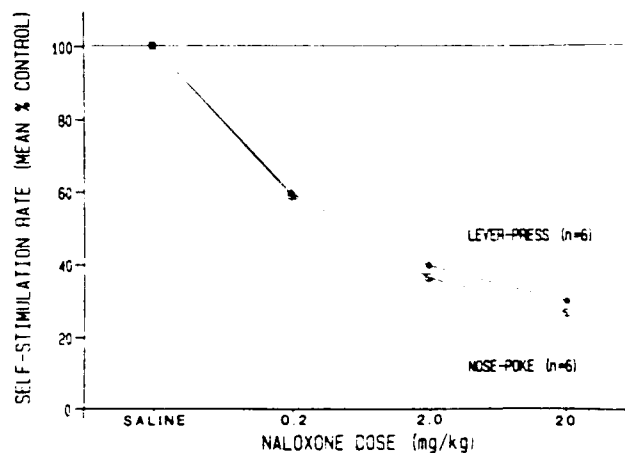


FIG. 8. Effects of naloxone on nose-poking and lever-pressing for nucleus accumbens self-stimulation for animals tested on nose-poke first, then switched to lever-press. Data points represent mean percent control ± standard error ($n=6$). Naloxone significantly suppressed self-stimulation for each task at all doses examined (Nose-poke: 0.2 mg/kg, $58.8 \pm 11.0\%$ of saline control, $p<0.001$ different from saline control; 2.0 mg/kg, $36.4 \pm 15.2\%$, $p<0.01$; 20 mg/kg, $26.2 \pm 7.4\%$, $p<0.01$; Lever-press: 0.2 mg/kg, $59.6 \pm 10.6\%$, $p<0.02$; 2.0 mg/kg, $39.4 \pm 8.1\%$, $p<0.05$; 20 mg/kg, $29.9 \pm 11.0\%$, $p<0.05$). There were no significant differences between the two tasks at any dose.

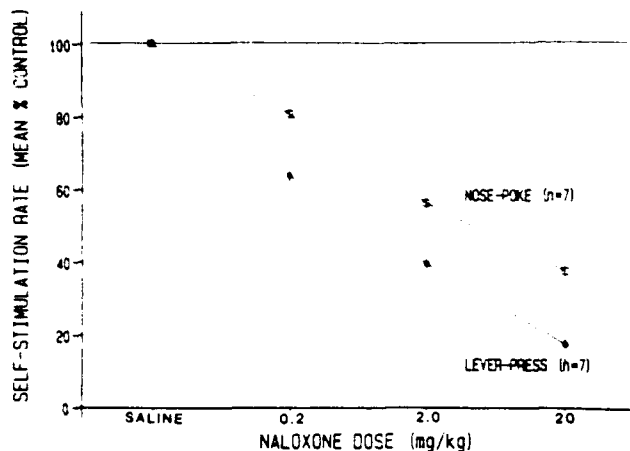


FIG. 9. Effects of naloxone on nose-poking and lever-pressing for nucleus accumbens self-stimulation for animals tested on lever-press first, then switched to nose-poke. Data points represent mean percent control \pm standard error. Naloxone significantly suppressed responding at all doses except the 0.2 mg/kg dose for nose-poking (Nose-poke: 0.2 mg/kg, $80.6 \pm 7.4\%$ of saline control, n.s. different from saline control; 2.0 mg/kg, $56.4 \pm 5.8\%$, $p < 0.01$; 20 mg/kg, $37.7 \pm 8.9\%$, $p < 0.001$). Lever-press: 0.2 mg/kg, $64.0 \pm 7.6\%$, $p < 0.01$; 2.0 mg/kg, $39.4 \pm 7.4\%$, $p < 0.01$; 20 mg/kg, $17.5 \pm 4.6\%$, $p < 0.01$). There were no significant differences between the two tasks at any dose.

nose-poking. It appears at the lower doses, that the effects of naloxone are not the result of a performance deficit. The difference between the tasks observed at the highest dose, however, suggests that in addition to suppressing reinforcement, at this dose naloxone may also have effects on motor capacity. These findings are consistent with studies demonstrating that suppression of locomotor activity by naloxone occurs only at doses of 10 mg/kg or greater (1, 8, 20, 21).

In the within-group comparisons in Experiment 2, differences in control response rate were dependent upon the direction animals were switched. Despite the fact that current intensities were not changed, animals that were switched from lever-press to nose-poke showed a large increase in control rate after the switch, while those that were switched from nose-poke to lever-press showed only a slight increase. These differences in response rate were reflected in the effects of naloxone on the two responses. When animals were switched from nose-poke to lever-press, the effects of naloxone on self-stimulation were virtually identical for the two tasks at all doses examined. However, when animals were switched in the opposite direction, from lever-press to nose-poke, naloxone was slightly less effective in suppressing nose-poke. The differences in sensitivity to naloxone in the lever-press to nose-poke group may have therefore resulted from the large difference in response rates between the two tasks for this group. This suggestion is supported by the observation that the group of animals that had only a small change in rate (those switched from nose-poke to lever-press) showed equal effects of naloxone on both tasks. As noted above, it is important to have equivalent baseline responses rates when comparing different tasks in self-stimulation. Reasons for the increase in rate observed in the lever-press to nose-poke group are probably two-fold: 1) the decreased motor output required by these animals on the nose-poke task allowed for greater response rates (16), and 2) a normal increase in response rate over time is typically observed in animals working for nucleus accumbens self-stimulation (19,35; Trujillo, unpublished observations). In the nose-poke to lever-press group, the greater difficulty of the lever-press task apparently countered

the normal increase in rate over time, resulting in only slightly higher response rates.

It is important to note that methodological factors may play a role in the ability of lever-press and nose-poke to distinguish between reward and performance effects in self-stimulation. While Gerhardt and Liebman (16) observed that the dopamine antagonist haloperidol suppressed nose-poking and lever-pressing for self-stimulation equally, Ettenberg, Koob and Bloom (11) observed that lever-pressing was more suppressed than nose-poking by the dopamine blocker alpha-flupenthixol. In the Ettenberg *et al.* study, which has been the subject of controversy (7), the authors tested the animals first on nose-poke, then on lever-press, using rate-intensity functions. In contrast, Gerhardt and Liebman (16) tested both operants in a single session, in a counterbalanced manner, examining response rates at a fixed current intensity. In the present experiments, we used a procedure similar to that of Ettenberg *et al.* (Experiment 1: testing animals on nose-poke first, then lever-press) as well as one similar to Gerhardt and Liebman (Experiment 2: counterbalanced testing, although we tested animals on only one operant per session). While we did not take into account all methodological factors (i.e., using fixed currents as opposed to rate-intensity function as Ettenberg *et al.*, and testing nose-poke and lever-press on different days as opposed to within a single session by Gerhardt and Liebman), the fact that we obtained similar results with both procedures suggests that the similar effects of naloxone on nose-poke and lever-press do generalize to different experimental situations.

The results of the present experiments, demonstrating that naloxone suppresses nose-poking and lever-pressing equally, are at odds with the conclusion drawn by West, Schaefer and Michael (46), that increasing the work requirements increases the ability of naloxone to suppress self-stimulation. In their study, West *et al.* utilized fixed ratio schedules in order to increase the work required by the animal to obtain a reinforcement, and observed that the higher ratio schedules were more suppressed by naloxone than lower schedules or continuous reinforcement. However, confounding the interpretation of West *et al.* is the fact that changes in the schedule of reinforcement also alter the density of reinforcement. With decreased reinforcing stimulations per unit time, there would be a concomitant decrease in the amount of endogenous opioids released, and less naloxone would be necessary to antagonize the behavior. Therefore, as alluded to by these investigators, the richness or density of reinforcement rather than the increased work, was more likely responsible for their effects. In the present studies, the three comparisons where the density of reinforcement (response rate) was closely matched, the effects of naloxone were equivalent for the two tasks. On the other hand, consistent with the study of West *et al.*, the one comparison in which the density of reinforcement was increased (increased response rate), the effects of naloxone were decreased. Thus, while our results are not inconsistent with those of West *et al.*, our experiments lead us to quite different conclusions about the role of work requirements in the ability of naloxone to suppress self-stimulation.

As noted above, drugs that interfere with the ability of the animal to respond should suppress lever-pressing for self-stimulation more strongly than nose-poking, while drugs that interfere with reinforcement should affect both responses equally. The present observation that naloxone suppresses nose-poking and lever-pressing equally is therefore consistent with the suggestion that opiate antagonists interfere with the reinforcement value of the brain stimulation reward rather than with the ability of the animal to respond. There are, however, other possible explanations for the similar effects of naloxone on nose-poke and lever-press. For example, naloxone may interfere with a portion of the response that is common to both tasks. Alternatively, this drug may produce sickness or aversion, thereby causing a generalized decrease in

responding. Thus, the present results do not prove that a motor deficit does not exist, nor do they provide direct support for a reinforcement interpretation. However, while the present studies do not completely rule out alternative explanations, a variety of studies support our suggestion that the suppression of self-stimulation is due to decreased reinforcement and not motor impairment, sickness, or other general debilitation. First, although naloxone has been observed to suppress locomotor activity, this effect requires doses of 10 mg/kg or greater (1, 8, 20, 21). In contrast, the observation in the present study, as well as in previous studies (2, 14, 32, 33, 39), that suppression of self-stimulation occurs at much lower doses suggests that motor effects are not responsible for the actions of naloxone on brain-stimulation reward. In a direct comparison of these behaviors, West, Schaefer and Michael (46) concluded that the modest effects of naloxone on locomotor behavior could not account for the suppression of self-stimulation. Second, if motor impairment, sickness or other nonspecific action were responsible for the suppressant effects of opiate antagonists, one would expect that all operant responding would be suppressed by these drugs. However, a variety of studies have demonstrated that naloxone can, in some experiments, facilitate responding (12, 18, 37, 45). Third, the observation that opiate antagonists have different effects at different self-stimulation brain sites (15, 17, 24, 25, 27, 32, 37), but see also (14) suggests that the actions of these drugs are site-specific, and not the result of a general suppression of behavior. Fourth, recent studies using threshold measures of self-stimulation (25, 44, 47) support our suggestion that naloxone interferes with the reinforcing value of the stimulation, rather than with the ability of the animal to respond. Finally, if the effects of opiate antagonists on self-stimulation were caused by sickness, aversion, motor-impairment or other nonspecific actions, then suppression of responding should be seen throughout the experimental session. However, our observation that opiate antagonists produce an extinction-like response decrement pattern in self-stimulation, with initial normal rates of response followed later by suppression (40), suggests that these compounds are indeed suppressing reinforcement rather than causing sickness, aversion or motor debilitation.

Despite the number of studies demonstrating significant suppression of self-stimulation by opiate antagonists, these effects remain controversial. Why do some studies observe effects of these drugs while others do not? Why do opiate antagonists typically only suppress self-stimulation rather than completely blocking this behavior? First, as noted above, careful examination of the studies that have used opiate antagonists in self-stimulation experiments reveals that methodology plays an important role in whether or not suppression is observed with these drugs—particularly important variables include electrode implant site and length of test session [see (40) for explanations of why these variables may be important]. Opiate antagonists do indeed suppress self-stimulation if appropriate methods are used. Second, although complete blockade of self-stimulation behavior by opiate antagonists has been observed (4), self-stimulation of most electrode sites is merely suppressed by these drugs. The most parsimonious explanation for these partial effects of opiate antagonists is that endogenous opioids do not play an exclusive role in self-stimulation. Endogenous opioids may be one of several neurotransmitters involved in this behavior—at some sites endogenous opioids may be of primary importance to the behavior; at some sites catecholamines may be of primary importance; and at other sites both endogenous opioids and catecholamines (as well as perhaps other transmitters) may contribute. Therefore, as would be expected, self-stimulation of some sites is completely blocked by opiate antagonists, self-stimulation of other sites is unaffected by these drugs, and self-stimulation of a third group of sites is suppressed, but not completely blocked.

In conclusion, the present study demonstrating that nose-poking and lever-pressing for self-stimulation are equally suppressed by the opiate receptor antagonist naloxone adds further evidence that motor debilitation is not responsible for the effects of opiate antagonists on self-stimulation. These results are consistent with the suggestion that opiate antagonists suppress self-stimulation by specifically blocking the reinforcing actions of stimulation-released endogenous opioids, and add to the increasing evidence that endogenous opioids may play an important role in reinforcement function (2, 5, 6, 10, 30, 36, 37).

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Pharmacology Biochemistry & Behavior

**PERTUSSIS TOXIN ATTENUATES INTRACRANIAL
MORPHINE SELF-ADMINISTRATION**

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ABSTRACT

Mu and delta opioid receptor subtypes are thought to mediate the reinforcing actions of opioids. These opioid receptors use PTX-sensitive inhibitory G proteins for signal transduction. Here we tested whether PTX would block the opioid reinforcement signals produced by intrahippocampal or intra-VTA injections of morphine in rats. Hippocampal PTX pretreatment prevented the acquisition of intrahippocampal morphine self-administration. Similarly, in rats previously trained to self-administer morphine in the VTA, PTX injected in the VTA abolished morphine self-administration behavior; the same PTX injections did not reduce responding reinforced by food pellets, suggesting that the toxin acted selectively to block morphine reinforcement rather than to generally interfere with motor capacity. Inactivated PTX did not reduce VTA morphine self-administration, indicating that the PTX blockade of opioid reinforcement is due to enzymatic inactivation of inhibitory G proteins. We concluded that inhibitory G proteins in the hippocampus and VTA may mediate the reinforcing effects of opioid drugs.

Opioid drugs of abuse are thought to produce their reinforcing effects by an activation of mu and/or delta opioid receptors in reward-relevant brain regions (for review, see Self & Stein, 1992). This conclusion is supported by experimental data from three reward paradigms. Selective agonists acting at mu and delta receptors supported self-administration behavior, enhanced the reinforcing value of subthreshold electrical brain stimulation, and clearly induced a conditioned place preference; conversely, central application of mu- or delta-selective antagonists effectively blocked both intracranial opioid self-administration and opioid-induced conditioned place preference. On the other hand, the kappa selective agonist U50,488 was not self-administered, and it failed to facilitate brain stimulation reinforcement or produce a place preference.

Mu and delta opioid receptors are known to modulate brain cell activity (Aghajanian & Wang, 1986; Crain et al., 1987; Dunwiddie & Su, 1988) and inhibit cyclic AMP formation (see Childers 1991) in a pertussis toxin (PTX)-sensitive manner. PTX irreversibly antagonizes opioid responses by a PTX-catalyzed ADP-ribosylation (inactivation) of inhibitory guanine nucleotide binding proteins (termed G_i and G_o) that mediate opioid receptor signal transduction (see Gilman, 1987). Injection of PTX in cerebral ventricles or appropriate brain regions can block morphine analgesia, an indication that central G_i/G_o proteins play a role in opioid antinociception (Hoehn et al., 1988; Sanchez-Blazquez & Garzon, 1988; Bodnar et al., 1990; Parolaro et al., 1990; Chang et al., 1991). We used a similar approach -- injection of PTX in the same brain sites that support intracranial morphine self-administration -- in an attempt to demonstrate involvement of G_i/G_o proteins in opioid reinforcement. One advantage of this approach is that the generalized toxicity of PTX is reduced because the treatments are confined to the brain region where the morphine is self-administered.

Rats will self-administer morphine directly into the hippocampal CA3 region (Grauer et al., 1989), and the ventral tegmental area (VTA; Bozarth & Wise, 1981). These experiments utilized an electrolytic microinfusion transducer system (EMIT) system to deliver nanoliter volumes of drug solution into discrete brain regions (Bozarth and Wise, 1980). The system utilizes electrolysis

of H₂O within a sealed reservoir to produce hydrogen and oxygen gas bubbles; these bubbles expel nanoliter volumes of drug solution through an injection cannula directly into the brain of freely moving rats. In this study, the effects of PTX pretreatment on intra-hippocampal and intra-VTA morphine self-administration were studied using the EMIT system.

METHOD

Subjects

Naive male Sprague-Dawley rats (Charles Rivers) initially weighing 270–300 g were used for these experiments. The animals were individually housed following surgery, fed ad libitum, and kept on a 12-hour light-dark cycle (lights on at 7:00 am).

Surgery

Under equitiesin anesthesia (3.33 ml/kg i.p.), animals were stereotaxically implanted with a unilateral guide cannula (22 gauge, Plastics One, Roanoke, VA) aimed at either the dorsal CA3 region of the hippocampus or the ventral tegmental area (VTA). Coordinates for surgery were: (CA3) –4.0 mm AP from bregma, 4.0 mm LAT, 3.1 mm ventral to dura, and (VTA) –5.0 mm from bregma, 0.8 mm LAT, 7.3 mm ventral to dura (Paxinos and Watson, 1982). Between tests, dummy cannulas extending 0.1 – 0.2 mm beyond the guide cannula tip were placed in guide cannulas. Animals were allowed a minimum of one week to recover from surgery before testing.

Apparatus

The injection cannula/reservoir (28 gauge, Plastics One, Roanoke, VA) was cut to extend 0.5 mm beyond the guide cannula tip. The injection cannula/reservoir was filled with drug solution and secured to an electrode/connector assembly (Plastics One, Roanoke, VA). Parafilm was wrapped tightly around the reservoir-electrode joint to insure an air-tight seal. The injection cannula was inserted into the implanted guide cannula and secured. The animal was then placed in a sound-attenuating experimental chamber (27 x 25 x 30 cm) containing two nose-poke holes.

Responses at the nose-poke holes were detected by photoelectric cells. The active nose-poke hole was indicated by a white cue light located just above the hole. A nose-poke response at the active hole activated the infusion of 100 nl (\pm 10 nl) of drug solution over 5 s, concurrent with a tone as a secondary reinforcer. The injection period was followed by a 30-s time-out period during which responses had no programmed consequences, and the cue light was extinguished. An inactive (non-reinforced) nose-poke hole was located on the same wall 7 cm adjacent to the active hole. A limit of 40 injections/8-h session was established to minimize brain damage due to excessive injection volumes. The system was controlled by a NOVA 4 computer (Data General, and Interact[®] I/O panel (BRS/LVE, Lexington, MA).

Procedure

Experiment 1: PTX pretreatments were injected via guide cannula in the CA3 region 2–3 days prior to both the first and third acquisition tests. Two separate PTX injections were given to insure that the PTX effect persisted for all three acquisition tests. The two PTX pretreatments were given under light methoxyurethane anesthesia to restrained animals; the 28 gauge injection cannula extended 0.5 mm beyond the tip of the guide cannula, and was connected by polyethylene tubing to a 1- μ l syringe (Unimetrics, Shorewood, IL). Each 100-ng dose of PTX was dissolved in 0.5 μ l phosphate-buffered saline and manually injected in 0.1 μ l volumes over 5 minutes. Control animals were injected with the buffered saline vehicle.

The effect of PTX on the acquisition of intracranial morphine self-administration was evaluated in CA3-implanted animals. Training was conducted during the light cycle in 3 8-h test sessions spaced 2 or 3 days apart. One animal failed to respond at the nose-poke hole and was eliminated from the study.

Six CA3-implanted animals used in self-administration experiments were simultaneously tested for PTX-induced changes in motor performance. Spontaneous locomotor activity in a 1 m x 1 m open field was tested 4–5 days following the initial PTX or vehicle pretreatment and within 1–2 days of the second self-administration test. The open field apparatus was divided into 25

sections, and the number of crossings in a 10-min test period was determined by an observer blind to the experimental condition.

Experiment 2: The effects of PTX on the maintenance of morphine self-administration was studied in VTA-implanted animals tested during their dark cycle. All animals used in this experiment first had to demonstrate reliable morphine self-administration in the VTA (at least 20 self-injections per test session). After baselines stabilized, a single 500-ng PTX injection (1 ul over 5 minutes) was injected in the VTA. After allowing 2 days for the toxin to take hold, morphine self-administration tests were again administered with 2-3 days between each test. Control animals received identical treatment except that heat-inactivated pertussis toxin was substituted for the active toxin.

Six of the VTA-implanted animals were concurrently tested for possible PTX-induced changes in response competence. These animals had received prior lever-press training with food pellets (45 mg, Bioserv) as the reinforcer. Immediately following self-administration testing, these animals were food-deprived for 24 h, and the number of food-reinforced lever presses/10 minutes was measured. The animals were then fed ad lib for at least 24 h prior to the next self-administration test session. Food reinforcement rates before and after PTX injections were compared.

Drugs

Morphine sulfate was dissolved in Ringer's solution (10 pmoles/100 nl injection) for hippocampal experiments. To control for pH changes with the higher morphine concentrations used in VTA experiments (300 pmoles/100 nl), morphine sulfate was dissolved in phosphate-buffered saline (308 mOs, pH = 7.4), containing 1.9 mM $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 8.1 mM $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 2.4 mM KCl, 137.6 mM NaCl. Pertussis toxin (salt free, List Biological Labs, Inc., Campbell, CA) was reconstituted and stored in phosphate-buffered as a stock solution at 0.5 ug/ul.

Histology

At the end of experiments, animals were injected with chloral hydrate anesthesia (5 ml/kg 0.526 M in 0.9 % saline, i.p.) and transcardially perfused with 0.9 % saline followed by 10 % formalin in saline. Brains were dissected, frozen and sliced in 40 μ m coronal sections. The sections were placed on gelatin coated slides, stained with cresyl violet, and examined for cannula placement. Only animals with accurate placements were considered for data analysis.

Data analysis

Daily self-administration totals from the hippocampal acquisition experiments were analyzed by two-factor ANOVA (Group x test session) with repeated measures on test session. Individual mean comparisons were conducted with Newman-Keuls' post hoc analysis. For vIA maintenance experiments, self-administration totals or food reinforcement rates were summed for three consecutive test sessions prior to (baseline) and following PTX pretreatment; the 3-day totals from before and after PTX pretreatment were analyzed using paired t-tests. An alpha level of 0.05 or less was considered statistically significant.

RESULTS

Table 1 shows various effects of 2 dose levels of PTX injected directly into the CA3 region of the hippocampus. Tonic-clonic type seizure activity developed in all animals pretreated with the high PTX dose (two 1- μ g injections spaced 6-7 days apart); these animals also exhibited a lack of grooming and marked weight loss. Three of the 6 high-dose animals died 3-5 days after the second injection. When the injected dose of PTX was reduced by an order of magnitude (0.1 μ g), no obvious behavioral deficits were noted. Furthermore, these animals had similar scores to vehicle-pretreated controls in open field tests for spontaneous locomotor activity. These and additional animals pretreated with the low PTX dose were tested for acquisition of intrahippocampal morphine self-administration in experiment 1.

Fig. 1 shows the acquisition of morphine (or Ringer's solution) self-administration in CA3-implanted animals on the first day of testing in experiment 1. The group pretreated with intrahippocampal PTX self-administered morphine at the same low rate as the control group that self-administered Ringer's solution. On the other hand, rats pretreated with the PTX vehicle quickly learned to self-administer morphine at a higher rate than the other groups throughout the test session. PTX pretreatment prevented the acquisition of morphine self-administration in each of the 3 acquisition tests (Fig. 2); again, the vehicle-pretreated group self-administered morphine at a higher rate than either the PTX-pretreated morphine group or the Ringer's control group ($F_{2,10} = 5.181$, $p = .029$). Thirteen of the 15 CA3-implanted animals were found to have accurate cannula placements in the CA3 region of the dorsal hippocampus. Two animals with cannula placements in the lateral ventricle were eliminated from the data analysis. The location of the injection cannula tips of the 13 animals retained in the experiment is shown in Fig. 3.

In experiment 2, VTA-implanted animals were first trained to reliably self-administer morphine sulfate (300 pmols/100 nl injection). Substitution of phosphate-buffered saline for the morphine reinforcement led to diminished self-administration (from 77.5 ± 9.47 responses in 3 morphine sessions to 36.7 ± 6.90 responses in 3 saline sessions; $T_5 = 10.877$, $p < .001$) (Fig. 4). Pretreatment with 500 ng PTX similarly reduced morphine self-administration from 61.1 ± 11.5 responses in 3 pre-PTX sessions to 43.5 ± 11.8 responses in 3 post-PTX sessions ($T_{10} = 3.174$, $p = .01$). Six of the PTX-treated animals were concurrently tested (on alternate days) for response competence at a food-reinforced lever. PTX did not significantly affect food-reinforced response rates lever-pressing rates (222.3 ± 19.1 responses in 3 pre-PTX sessions and 197.0 ± 27.5 responses in 3 post-PTX sessions) ($T_5 = 1.424$, $p = .214$). Morphine self-administration thus was significantly reduced by PTX at the same time that food-reinforced responding was not. Animals pretreated with heat-inactivated PTX self-administered morphine at a rate similar to their prior baseline self-administration rates (62.3 ± 17.8 responses in 3 pre-PTX sessions vs. 68.3 ± 17.8 responses in 3 post-PTX sessions) ($T_3 = .399$, $p = .717$).

Three of the 15 rats that began experiment 2 had to be discarded. One animal with a correctly-placed VTA cannula failed to demonstrate reliable morphine self-administration; a second rat with a misplaced cannula approximately 1 mm lateral and 1 mm dorsal to the target site also did not self-administer morphine above saline levels, and a third rat dislodged its guide cannula before the experiment was completed. The cannula placements of the remaining 12 animals used in the data analysis are shown Fig. 5.

DISCUSSION

Pretreatment with intrahippocampal PTX prevented the acquisition of morphine self-administration in the CA3 region of the hippocampus. Similarly, VTA injections of PTX reduced morphine self-administration to saline control levels in animals previously trained to self-administer morphine into the VTA. The toxin produced no obvious motor or performance deficits at the doses employed in the self-administration experiments. Indeed, in the VTA-treated animals, PTX selectively reduced nose-poke responses for morphine injections while failing to reduce higher rates of lever-pressing for food reinforcement. Since significant effects of PTX on motor performance can thus be excluded, the reduction in morphine self-administration produced by the toxin is more reasonably explained by blockade of morphine reinforcement.

Morphine exhibits a marked preference for mu opioid receptors (Kosterlitz, 1987). Stevens et al. (1991) found that dynorphin A self-administration in the hippocampus was attenuated by co-infusion with a mu-selective but not delta- or kappa-selective antagonists, and Devine & Wise (1990) reported VTA self-administration with mu- and delta-selective agonists. Thus, morphine reinforcement may result from activation of reward-relevant mu opioid receptors in the hippocampus, and both mu and delta receptors in the VTA. The present findings with PTX are consistent with this interpretation, since both mu and delta receptor-mediated responses are blocked by the toxin (see Childers, 1991). Kappa-mediated opioid responses, which are not

blocked by PTX in rat membranes (Childers, 1991), also do not mediate opioid reinforcement (see Self & Stein, 1992).

The hippocampus displayed greater sensitivity than the VTA to the reinforcing effects of morphine, as exemplified by a 30-fold difference in effective dose. Receptor-mediated responses in the hippocampus may be intrinsically more sensitive to morphine, or the difference in sensitivity may be explained by differences in the density or distribution of mu opioid receptors in the two regions (Mansour et al., 1987). Lower doses of PTX also were required to block morphine in the hippocampus, but, as noted, the dose of morphine to be surmounted was much lower.

The few animals with misplaced guide cannulas did not acquire self-administration behavior; hence it is likely that the morphine acted at the targeted CA3 and VTA sites rather than elsewhere. This suggestion is supported by earlier reports that opioid self-administration in the CA3 (Stevens, et al., 1991) and the VTA (Bozarth, 1983) has anatomical specificity. It also seems unlikely that the PTX produced its effects in nontargeted brain regions since intracerebral PTX injections remain highly localized (Van der Ploeg et al., 1991). Moreover, a more widespread effect of intracerebral PTX would probably also have reduced response rates for food reinforcement, and this was not the case.

Inactivated PTX failed to alter morphine self-administration, indicating that enzymatic activity is required for PTX to produce its reinforcement-blocking effects. Since G_i and G_o proteins are the only known substrates for PTX in brain (Neer et al., 1984; Sternweiss & Robishaw, 1984), it is likely that their inactivation was responsible for the attenuation of morphine's action at the injected brain site. If so, our results can be taken to indicate that G_i and/or G_o -mediated pathways are involved in opioid reinforcement. This conclusion is additionally supported by recent evidence that PTX pretreatment prevents the acquisition of conditioned place preferences induced by mu and delta opioid agonists (Suzuki et al., 1991).

It may be argued that our results with PTX could be due to a more generalized disruption of neurotransmission in the hippocampus and VTA, than to specific impairment of opioid signal transduction. For example, opioids are thought to produce their reinforcing effects in the VTA via activation or disinhibition of VTA dopamine neurons (Leone et al., 1991; Johnson & North, 1992). Since dopamine neurons possess autoreceptors which are blocked by PTX (Innis & Aghajanian, 1987), and chronic blockade of these autoreceptors can result in depolarization inactivation of dopamine neurons (Bunney & Grace, 1978; White & Wang, 1983), PTX could block morphine self-administration by inactivation of dopamine neurons rather than by impairment of opioid receptor transduction. However, the same PTX dose used in our studies has been reported to enhance cocaine's facilitation of dopamine release from VTA neurons (Steketee et al., 1991), an observation inconsistent with PTX-induced inactivation of VTA dopamine neurons. If so, the present finding that PTX attenuates morphine reinforcement is more likely due to the specific uncoupling of opioid receptors from their signal transduction mechanisms than to generalized disruption of VTA neurotransmission. Similarly, in the hippocampus, PTX injections are reported to block the electrophysiological effects of mu and delta opioid agonists without affecting the functional integrity of downstream pathways (Dunwiddie and Su, 1988). Again, this finding supports the conclusion that PTX attenuated opioid reinforcement in the hippocampus by blocking specific opioid receptor-mediated processes, rather than by nonspecific blockade of hippocampal neurotransmission.

In conclusion, local injections of PTX attenuated intracranial morphine self-administration in both the hippocampal CA3 region and VTA. Control experiments indicated that the PTX-induced attenuation involved inactivation of G_i and G_o proteins, and was produced by reward-related and not performance-related changes. These findings suggest that G_i and/or G_o proteins in the hippocampus and VTA mediate the reinforcing effects of opioid drugs.

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Table 1. Observation of various behavioral effects of pertussis toxin after injected into the CA3 region of dorsal

| Condition* | N | Wt. Gain (g) (\pm SEM) | Activity [†] (\pm SEM) | Grooming | Seizures | Deaths |
|---------------|---|------------------------------|---------------------------------------|----------|----------|--------|
| Vehicle | 3 | 55 \pm 5 | 232 \pm 42 | yes | None | None |
| PTX 0.1 ug | 3 | 57 \pm 10 | 245 \pm 41 | yes | None | None |
| PTX 1.0 ug | 6 | wt. loss | NT | no | 6 / 6 | 3 |

*Two injections were given 3 or 6 days apart.

[†]Activity was scored in an open field for 10 min 4 days after the first injection (NT=not tested).

FIGURE LEGENDS

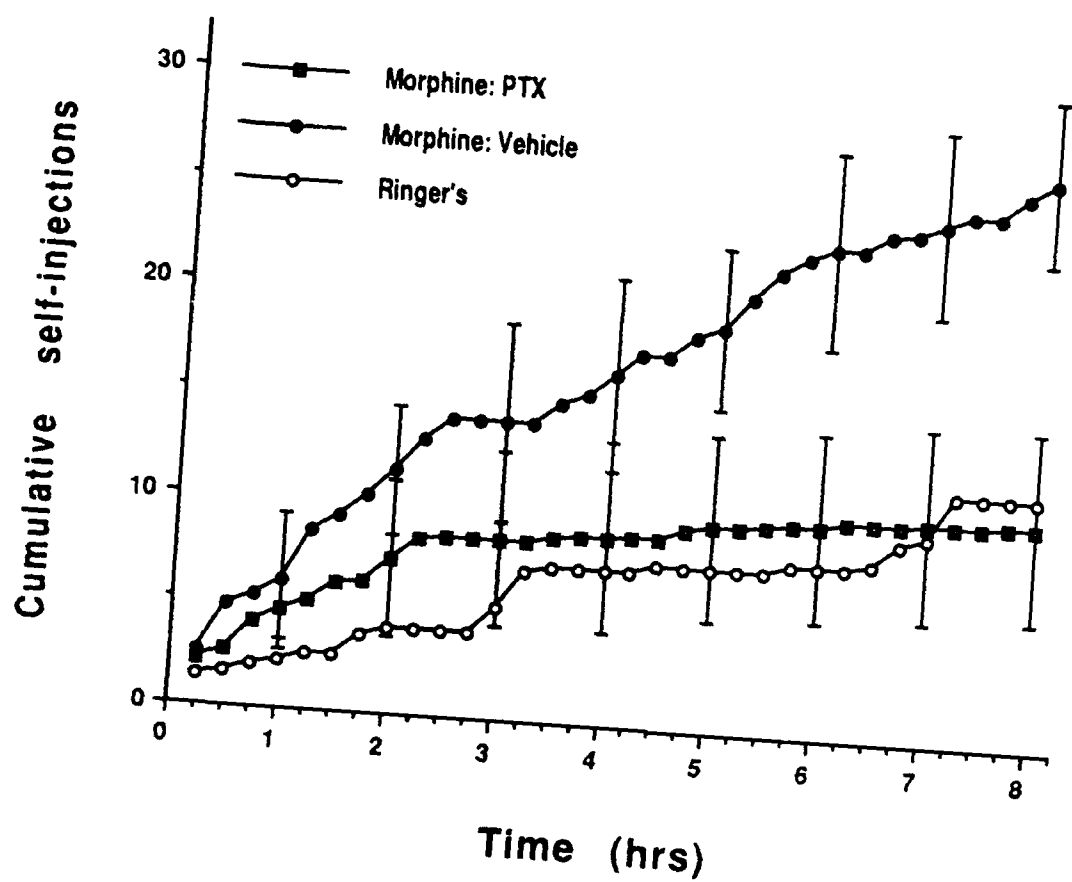
FIG 1. Effects of pertussis toxin (PTX) pretreatment on acquisition of intrahippocampal morphine self-administration on the first test day. Data points show the mean (\pm S.E.M.) cumulative self-injections over the 8-h test session. Six rats self-administering morphine sulfate (10 pmole/100 nl injection) were pretreated intrahippocampally with PTX (Morphine: PTX). Four rats self-administering morphine were pretreated with the PTX vehicle (Morphine: Vehicle). A second control group received no hippocampal pretreatment and was reinforced only with Ringer's solution ($n = 3$).

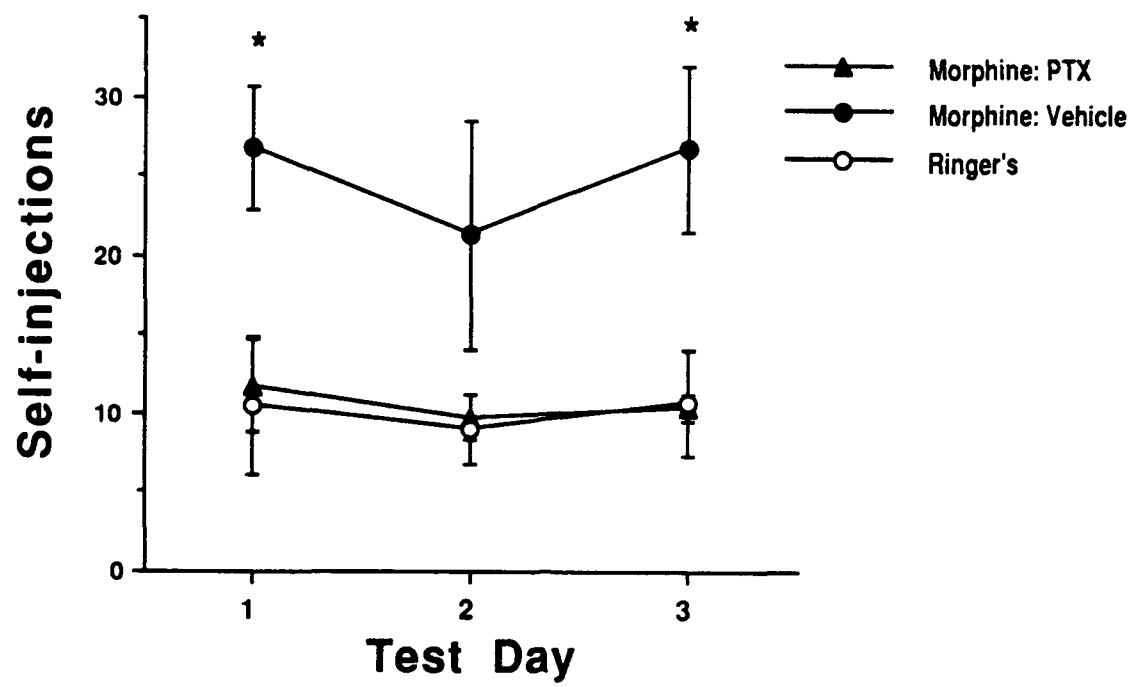
FIG 2. Effects of pretreatment with PTX (100 ng) on acquisition of morphine self-administration in the CA3 region of hippocampus for each of 3 test sessions. Data are expressed as the mean (\pm S.E.M.) self-administration totals for each test sessions. Groups consisted of animals self-administering morphine (10 pmol/100 nl injection) and pretreated with PTX (Morphine + PTX) or vehicle (Morphine + Vehicle), and controls self-administering Ringer's solution. * signifies significant differences from both Morphine + PTX, and Ringer's groups ($p < .05$, Newman-Keuls).

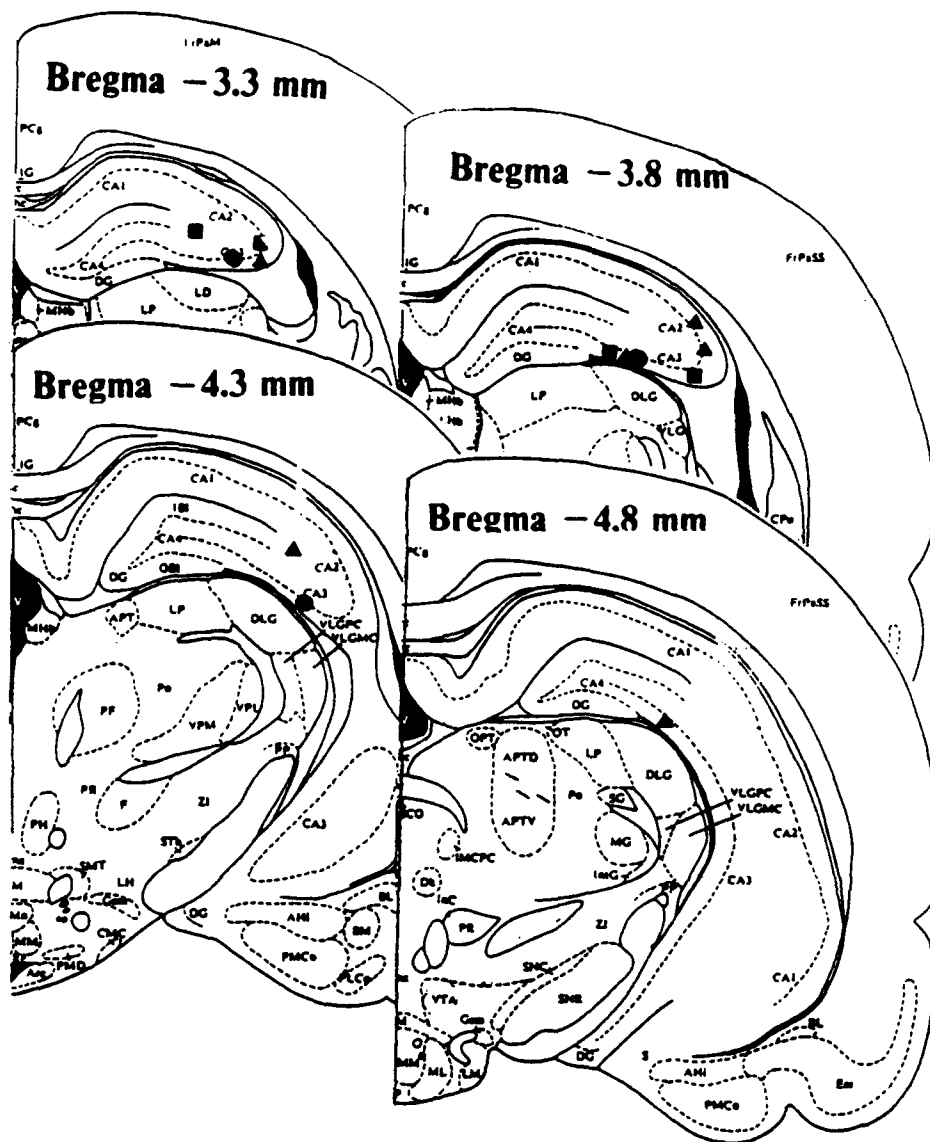
FIG 3. Localization of injection cannula tips in the CA3 region of dorsal hippocampus for the thirteen animals used in data analysis. The three groups are indicated as follows: Ringer's (circles), Morphine + Vehicle (squares) and Morphine + PTX (triangles).

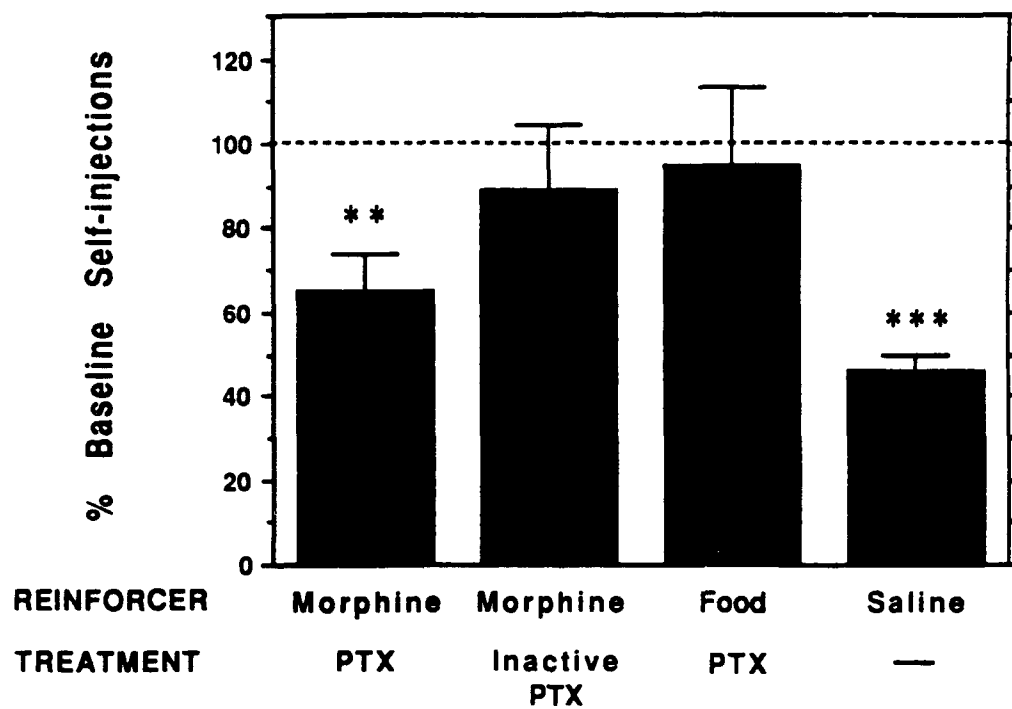
FIG 4. Effects of pretreatment with 500 ng PTX ($n = 11$) on the maintenance of VTA morphine self-administration (300 pmol/100 nl injection) or food-reinforced behavior ($n = 6$). Controls show the effects on VTA self-administration of pretreatment with inactive PTX ($n = 4$) or substitution of saline for morphine ($n = 6$). Data are expressed as the mean (\pm S.E.M.) of % baseline responding (see text). * signifies $p = .002$; ** $p < .001$ (paired T-test) when compared to baseline responding.

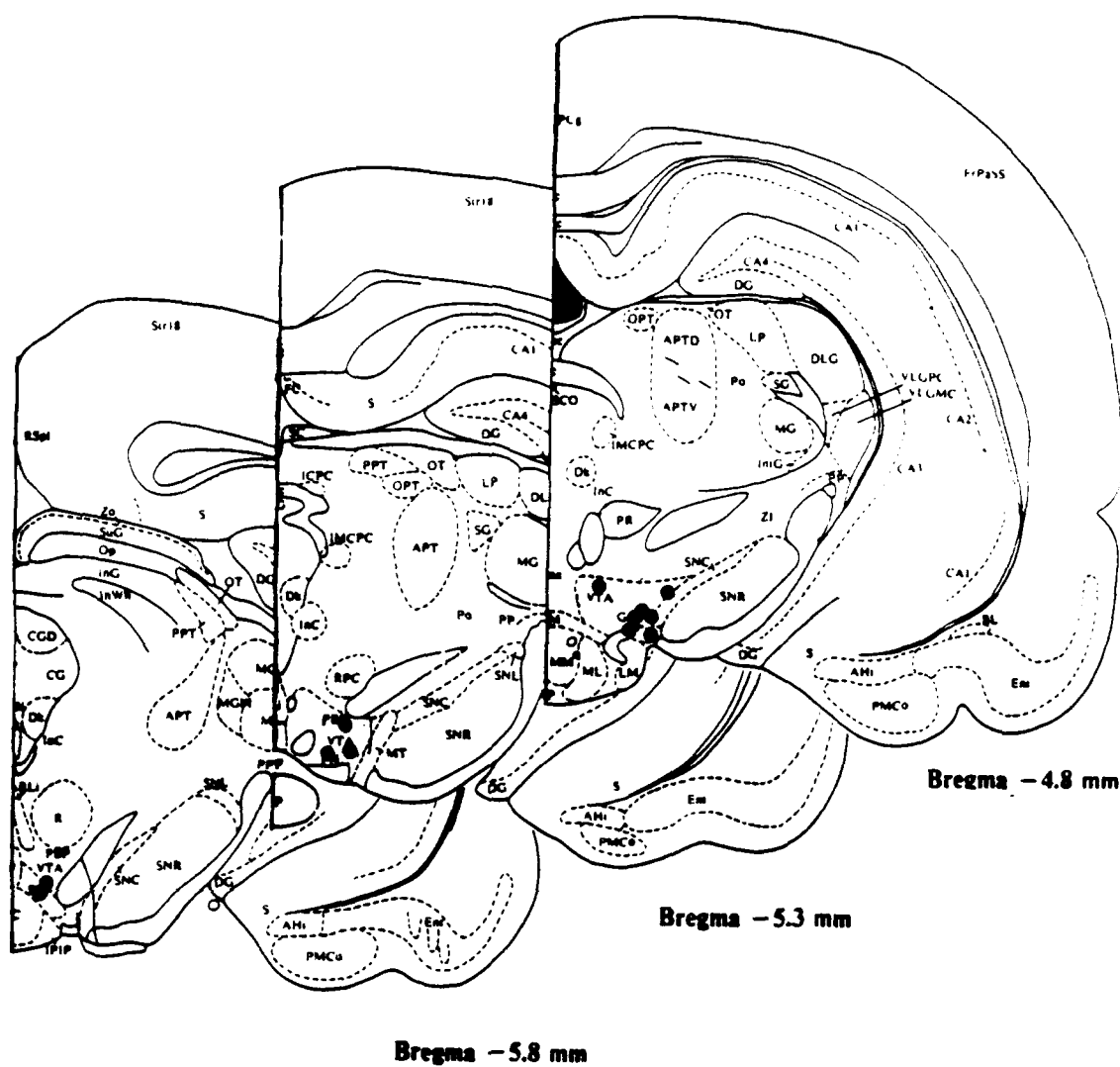
FIG 5. Localization of injection cannula tips in the VTA region for the twelve animals used in the data analysis. All animals exhibited reliable morphine self-administration behavior prior to the PTX treatments.











-- PRELIMINARY DRAFT --

OPPOSITE ACTIONS OF DOPAMINE AND GLUTAMATE IN
OPERANT CONDITIONING OF HIPPOCAMPAL BURSTING

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ABSTRACT

Previous work indicates that hippocampal CA1 cell bursting activity may be reinforced by local micropressure application of dopamine (1 mM). However, there is a concern that dopamine may act merely by direct or indirect pharmacological stimulation of bursting. One approach is to attempt to reinforce hippocampal bursting with a relatively nonspecific depolarizing agent such as glutamate to compare the reinforcing effect of it with dopamine. Unlike dopamine, burst-contingent applications of glutamate did not produce selective facilitations of cellular bursting when compared to dopamine presentations; indeed, both contingent and random glutamate applications reduced the likelihood of bursts, while at the same time greatly increasing the frequency of individual spikes. These results are consistent with the idea that dopamine's reinforcing action on hippocampal CA1 bursting can be attributed to specific stimulation.

Key Words: Reinforcement, Dopamine, Glutamate, Hippocampal CA1 Cell.

INTRODUCTION

In the mammalian CNS catecholamines are thought to play an important role in the pharmacological actions of psychomotor stimulants such as amphetamine and cocaine. Dopamine, in particular, appears to be involved in the reinforcing properties of psychomotor stimulants. Dopamine agonists have been shown to be self-administered by several species (12, 26, 29). Dopamine receptors are of two general types, D1-like and D2-like. Both subfamilies of dopamine receptors may have reinforcing effects; our own recent work with the full D1 agonist SKF 82958 indicates that activation of D1 receptors can reinforce both cellular and behavioral operant conditioning. The dopamine reinforcement hypothesis is also supported by experiments with dopamine antagonists (13, 15). It is also necessary to ask what are the neural targets of the reinforcing system? It is commonly believed that a behavioral response reflects the activity of many neurons. Is it the individual activities of the relevant neurons that is reinforced; that is, is positive reinforcement exerted at the cellular level? The theoretical work of Klopff (16) and the impressive explanatory power of current cellular models of classical conditioning (11) have led us to consider the possibility that individual neuronal activity may be modifiable by the activity-contingent action of reinforcing transmitters, such as dopamine (3). Our previous studies (21) revealed that the spontaneous bursting of individual CA1 pyramidal neurons was increased with locally applied activity-contingent injections of dopamine. It is important to demonstrate, however, that dopamine did not act by direct or indirect pharmacological stimulation or facilitation of bursting. The present study was designed to determine whether CA1 hippocampal bursting can be reinforced with activity-contingent application of the nonspecific depolarizing agent glutamate.

METHODS AND MATERIAL

The experiments were performed on transverse hippocampal slices prepared from male Sprague-Dawley rats (200-270 g). The rats were lightly anesthetized with Halothane and decapitated. The brain was removed rapidly from the skull and allowed to cool at 4 C in artificial cerebrospinal fluid (ACSF) containing NaCl (124 mM), KCl (5 mM), CaCl₂ (2.4 mM), MgSO₄ (2 mM), KH₂PO₄ (1.25 mM), NaHCO₃ (26 mM) and glucose (10 mM). The hippocampus was dissected out and sliced into 400- μ M slices using a McIlwain tissue chopper. Using an eyedropper, 6-8 slices were individually transferred to a static chamber where they were supported on nylon mesh at the surface of the ACSF solution in an oxygenated atmosphere (95% O₂, 5% CO₂) at 35 C. The ACSF solution in the static chamber was changed every 30 min, unless prohibited by potential disruption of an ongoing experiment. Following incubation for at least 2 hr, cellular activity was recorded using single-barrelled extracellular micropipettes filled with vehicle or drug solution and with the tip broken to permit pressure ejection of a 10 μ -diameter droplet following a 50-msec application of nitrogen at 15 P.S.I. During operant conditioning, micropressure injections of drug were applied directly to the cell for 50 msec following bursts of activity. Drug-induced increases in bursting are necessary but not sufficient evidence of cellular operant conditioning, since the drug treatments may directly stimulate or facilitate cellular firing. As a mandatory control for such pharmacological stimulation, the same drug injections must be administered independently of bursting on a noncontingent or random basis. Cellular reinforcing effects may be inferred only if the noncontingent injections are relatively ineffective. The experimental setup is shown diagrammatically in Fig 1. A burst was

defined as a train of firing containing N or more spikes with a maximum interspike interval (ISI) of t msec. Normally, reinforceable bursts of activity contained 3-6 spikes with a maximum ISI of 10 msec. The parameters were set individually for each test neuron such that bursts occurred at a baseline rate of approximately 5 per min.

A complete neuronal operant conditioning experiment involved six stages: Baseline: the rate of bursting prior to operant conditioning was determined in a baseline period of approximately 5-10 minutes. Reinforcement: each burst was now followed by an injection of the test solution. To minimize injection artifacts, neuronal activity during and for 3 sec after each injection was excluded from analysis and had no programmed consequences. Extinction: reinforcement was terminated and recording continued until the baseline burst rate was recovered. Matched (Free) Injections: noncontingent injections of the test solution were given at regular intervals to determine the direct pharmacological effects on neuronal activity. The number of injections was matched to the 3-5 highest injection rates received during the prior reinforcement period. Again, neuronal activity during and for 3 sec after each injection was excluded from analysis. Washout: a second baseline period was given in order to allow residual effects of drug administration to dissipate and for baseline burst rates to return. Reacquisition: a second period of reinforcement was scheduled, whenever possible, in order to compare rates of original acquisition and reacquisition and to ascertain the viability of the preparation following noncontingent injections.

RESULTS

Contingent applications of dopamine produced a significant reinforcing action on hippocampal CA1 neurons. A typical reinforcement experiment on rat hippocampal slice CA1 neurons is shown in Fig 2. It can be seen that in two separate periods of operant conditioning (REINF), the frequency of bursts and the overall firing rate were rapidly increased after several contingent applications of dopamine (1 mM). The same dose of dopamine injections administered noncontingently (MATCH) failed to increase either frequency or overall firing rate. This result is consistent with our previous finding. Unlike dopamine, contingent applications of glutamate (0.05, 0.1 and 0.2 mM) did not produce selective facilitation of cellular firing rate when compared to random presentations, indeed, in most experiments, both contingent and noncontingent applications of glutamate did not increase the likelihood of CA1 cell bursting, while at the same time increasing the frequency of individual action potentials and the overall firing rate (shown in table 1.). Fig 3 shown a typical experiment on rat hippocampal slice CA1 cells. Different significantly from dopamine, burst-contingent application of glutamate (0.1 mM) did not increase the cellular bursting but increasing single spike frequency of individual cells.

DISCUSSION

The objectives of these experiments were to examine the reinforcing effect of dopamine on hippocampal CA1 neuron bursting is specific, rather than not during to direct or indirect pharmacological stimulation of bursting by reinforcing hippocampal CA1 neuron with applications of glutamate and dopamine. The above results showed that contingent applications of dopamine induced a significantly reinforcing action on hippocampal CA1 cells. In contrast with dopamine, glutamate (0.05, 0.1 and 0.2 mM) did not increase hippocampal CA1 neurons bursting followed contingent application, while at the same periods greatly increasing the frequency of individual spikes rate (Fig 3). Glutamate is an excitatory amino acid which has been suggested to play a major role in the excitatory neurotransmission (7, 9, 10, 24). However, except for uptake mechanism, little is known about possible intrinsic mechanism to regulate effects of glutamate in the CNS. Some studies demonstrated that continuous bath perfusion of glutamate and its analogues in the rat hippocampal slice was shown to selectively and reversibly depress excitatory postsynaptic potentials (6, 17). Repeated exposure to glutamate elicits successively excitatory response accompanied by a parallel decrease in synaptic potentials (22). A recently reported that revealed that (1) fade of response to prolonged glutamate application in the rat hippocampal slice was linked during to the postsynaptic receptor desensitization. It is of interest that our data here demonstrates that glutamate failed to increase hippocampal CA1 neurons contingent bursting, whereas increasing the single spikes rate of CA1 neurons. The single spikes rate increasing of CA1 cells caused by glutamate may resulted from directly pharmacological stimulation, since glutamate also increase

hippocampal CA1 neuron single spike rate during Free reinforcement injection periods (MATCH) (Fig 3). On the other hand, the results of different doses of glutamate on the reinforcing action on hippocampal CA1 neuron contingent bursting is good control of dopamine' reinforcing action. The result suggest that the dopamine reinforcing the hippocampal CA1 cell contingent bursting may not simply due to directly pharmacological stimulation. Alternatively, the reinforcing action of dopamine on hippocampal CA1 neuron bursting is specific and appeared be mediated by D2 receptor (21). Ground on the different reinforcing effect of glutamate and dopamine as well as hippocampal CA1 cells bursting was not increased by noncontingent administration dopamine, we can rule out the possibility that direct stimulant effects of dopamine caused the increase in neuronal activity that were observed in the reinforcement periods. Since it is generally accepted that the inhibitory action of directly dopamine in the brain is more common than the excitatory one (19). Some previous studies have shown that application of dopamine to hippocampal slice can evoke either excitation or inhibition of CA1 pyramidal neurons, the effect being reflected, respectively, by an increase or decrease in their spontaneous firing rate. The excitation evoked by dopamine on hippocampal slice was due to activation of the D2 dopamine receptors, while the inhibition was the result of stimulation of the D1 dopamine receptor (19). In the pervious cellular operant conditioning experiments, we first found that the reinforcing action exerted by dopamine on hippocampal CA1 cells was mediated by dopamine D2 receptors (3, 21). These combined observations further support the hypothesis that dopamine D2 receptors may play an important role in either the reinforcing action or physiological function on hippocampal CA1 neurons. In conclusion, glutamate did not reinforce hippocampal slice CA1 contingent bursting, but both contingent and random

glutamate applications of increased the frequency of individual spikes. The effect of glutamate increasing hippocampal slice CA1 spike rate may be due to a direct or indirectly pharmacological stimulation. Dopamine differently from glutamate, increased significantly hippocampal CA1 cell bursting and overall firing rate after contingent applications of dopamine the same dose of dopamine injections noncontingently failed to increase hippocampal CA1 cell burst or overall firing rate. These data have added a strong evidence to our previous findings that dopamine may play a key role in operant conditioning of hippocampal CA1 neurons and dopamine's reinforcing action on hippocampal contingent bursting is specific.

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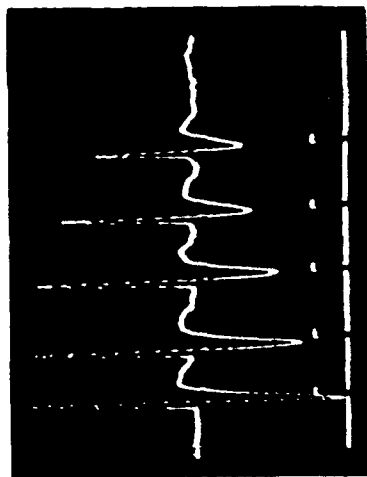
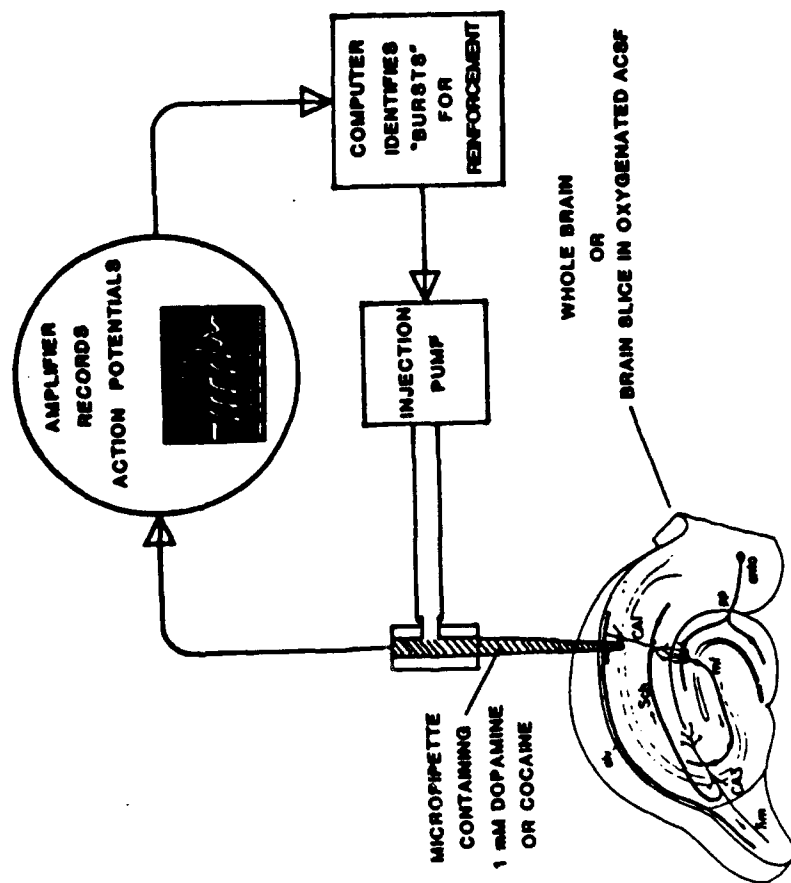
FIGURE CAPTIONS

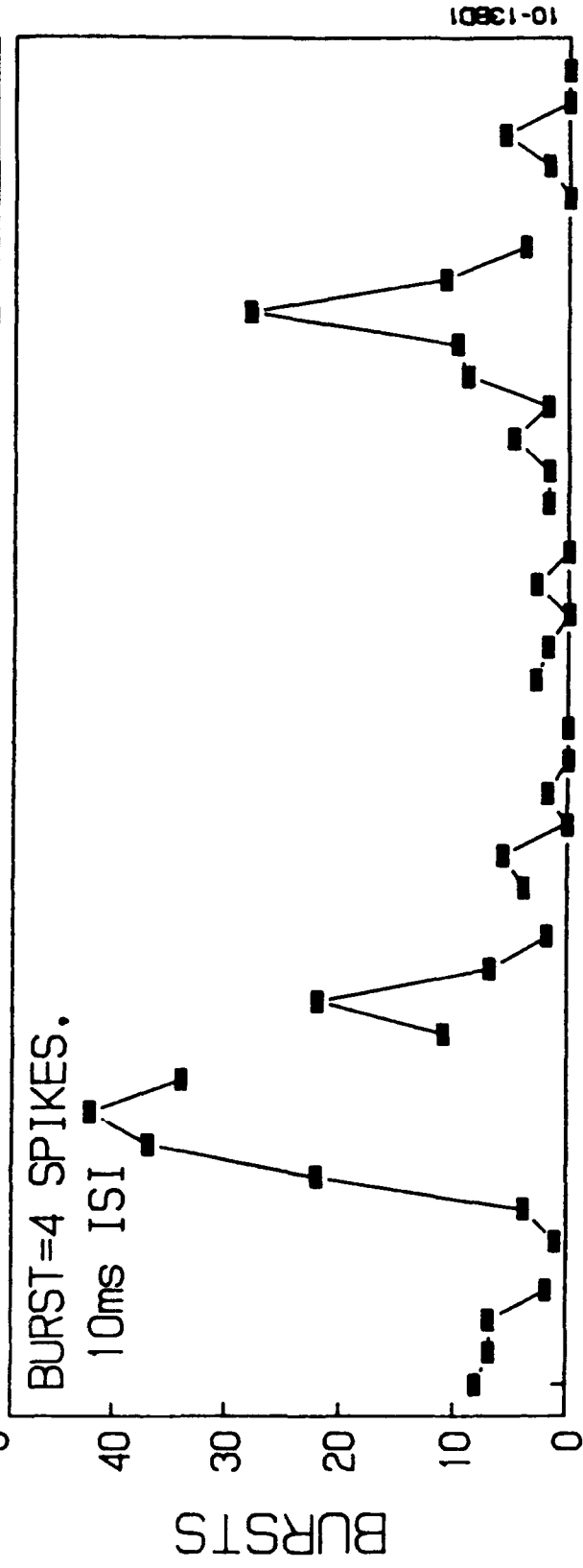
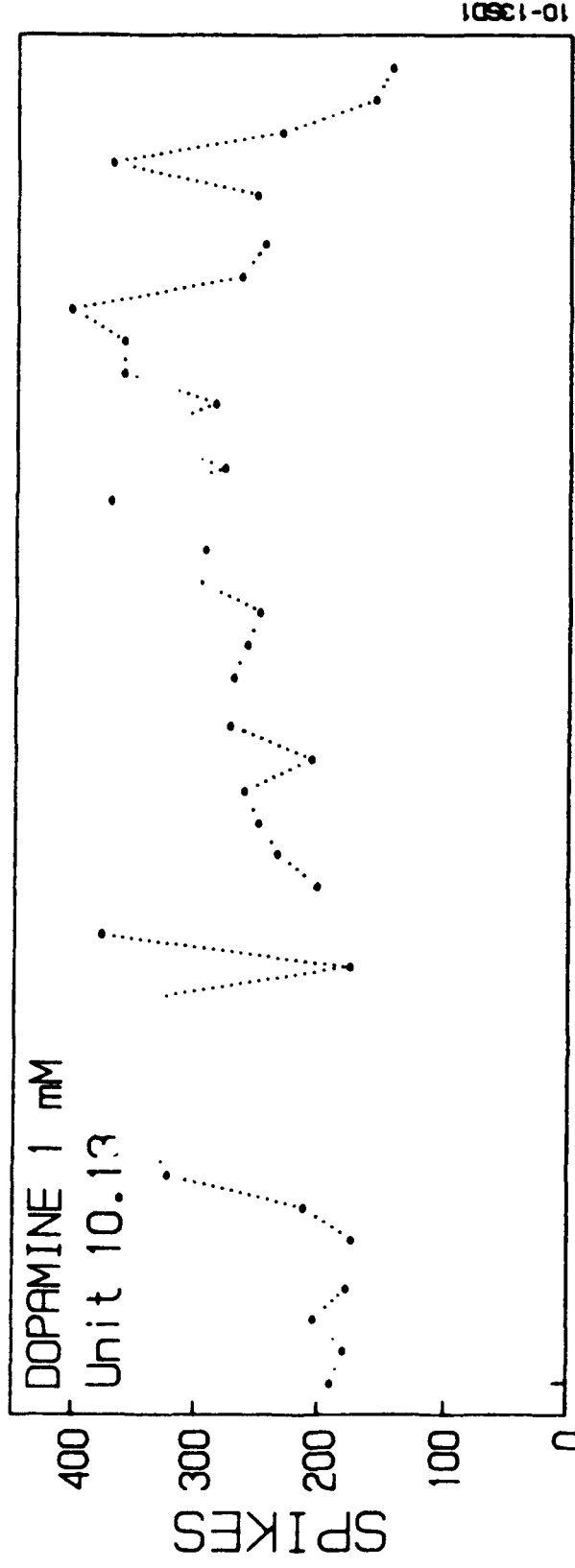
Fig 1. A. Schematic diagram of cellular operant-conditioning experiment. A single-barrelled glass micropipette for simultaneous recording and pressure injection is filled with dopamine (1mM in 165 mM saline) or other drugs and aimed at spontaneously active hippocampal cells in the CA1 layer. Amplified action potentials are processed by a spike enhancer and window discriminator (not shown) to increase the signal-to-noise ratio and to isolate signal when multiple-unit activity was encountered. When the computer recognized a reinforceable burst of activity (based on criteria established individually for each test neuron before operant conditioning), the pressure-injection pump was activated for 50 msec to deliver an approximately 10 μ m-diameter droplet of drug in the close vicinity of the cell. During-induced increase in bursting are necessary but not sufficient evidence of cellular operant conditioning, since the chemical treatments may directly stimulate or facilitate cellular firing. As a mandatory control for such pharmacological stimulation, the same drug injections are also administered independently of bursting on a noncontingent or "free" basis. Cellular-reinforcing effects may be inferred only if the noncontingent injections are relatively ineffective (28). B. (Upper trace) Burst of firing recorded extracellular from a CA1 cell, exhibiting typical decrescendo pattern with progressively shorter and broader spikes occurring later in the burst. (lower trace) 1-msec logic pulse. Spikes that satisfy the present criteria of the discriminator are converted to logic pulses for counting by the computer.

Fig. 2. Operant conditioning of the activity of a CA1 pyramidal cell in a slice of dorsal

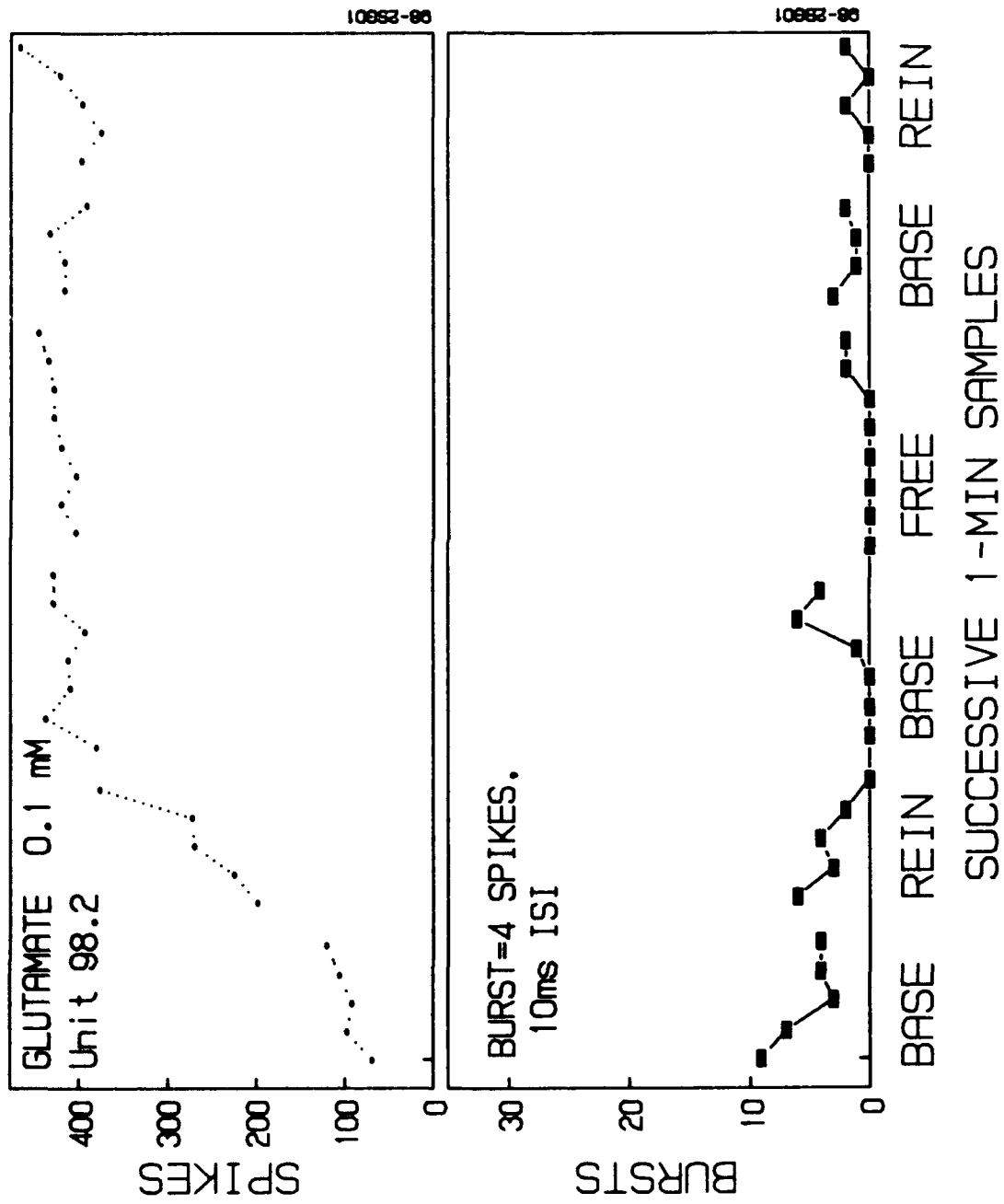
hippocampus with local injections of dopamine used as reinforcement. The activity of the unit through seven phases of a complete experiment is shown. Each point shows the number of bursts (lower graph) and the total number of spikes (Upper graph) in successive blocks of 100 half-second samples or trials. Prior to the first baseline phase, a burst criterion of 4 or more spikes per half-second sample was selected. This criterion gave a burst rate for this unit that never exceeded 4 percent in the initial baseline period (BASE). In the reinforcement period (REINF), dopamine HCL (1mM in 165 mM saline) was applied for 5 msec immediately after each burst. Following a second baseline period, the same dopamine injections were delivered (MATCH) independently of the unit's behavior as a control for possible stimulant effects. The number of injections was matched to that earned during the last four periods of the reinforcement phase. Rates of bursting and overall firing were increased by the contingent dopamine injections during the reinforcement periods, but were not increased when the same injections were administered noncontingently in the matched-injection period.

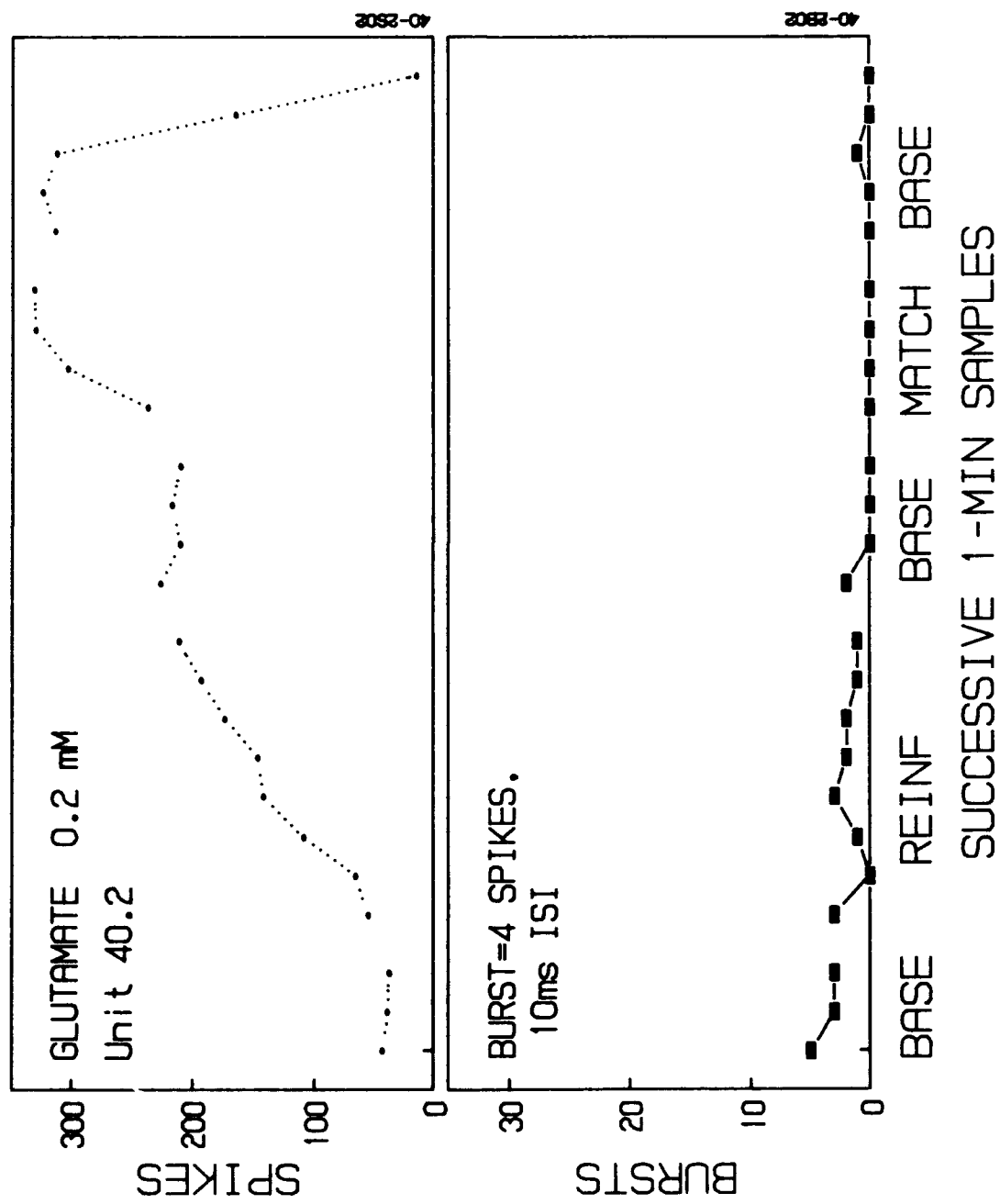
Fig.3. Operant conditioning of a CA1 pyramidal neuron in a dorsal hippocampal slice using local injections of glutamate (0.1 mM) as reinforcement. For details, see Fig 2. and Table 1. Number of bursts and spikes is calculated by averaging the two highest 100-trial (or 50-sec) bursting and spike scores recorded for each unit, and then averaging for the different group. ** $P < 0.01$ compared with saline; * $P < 0.05$ compared with saline.





BASE REINF BASE MATCH BASE REINF BASE
SUCCESSIVE 1-MIN SAMPLES





Naloxone blockade of amphetamine place preference conditioning*

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Abstract. Amphetamine and naloxone were examined in place conditioning, in order to study possible interactions between endogenous opioids and catecholamines in reinforcement. After initial preferences were determined, animals were conditioned with amphetamine alone (1.0 mg/kg SC), naloxone alone (0.02, 0.2 or 2.0 mg/kg SC) or combinations of amphetamine plus naloxone. A reliable, long-lasting preference for the compartment associated with amphetamine was observed, reflecting the reinforcing properties of this drug. No preference or aversion was observed in animals that received saline in both compartments. Naloxone (0.02, 0.2 and 2.0 mg/kg) produced a dose-dependent place aversion; while the lowest dose had effects similar to saline, the higher doses produced significant place aversions. Naloxone, at all three doses examined, prevented the ability of amphetamine to produce a place preference. Thus, the lowest dose of naloxone, having no effects alone in place conditioning was still able to block the reinforcing effects of amphetamine. These results suggest that the reinforcing effects of amphetamine are dependent on activation of opiate receptors, and provide further evidence that interactions between endogenous opioids and catecholamines may be important in reinforcement.

Key words: *d*-Amphetamine · Naloxone · Place conditioning · Conditioned place preference · Reward · Reinforcement · Endogenous opioids · Catecholamines

Evidence suggests that two types of neurotransmitter, catecholamines and endogenous opioids, may be important in the rewarding actions of drugs of abuse and other stimuli (Stein 1978; Watson et al. 1989). Catechol-

amines, particularly dopamine, appear to mediate the reinforcing properties of the psychomotor stimulants amphetamine and cocaine, while opiate drugs produce reinforcement by mimicking the actions of endogenous opioids at opioid receptors. Additionally, studies suggest that opioids and catecholamines, and the drugs that affect these systems, may interact in reward processes. Depletion of catecholamines with alpha-methyl paratyrosine prevents self-administration of morphine (Davis and Smith 1973) and suppresses the potentiating effects of morphine on self-stimulation (Pert and Hulsebus 1975). Dopamine receptor antagonists have been observed to block the reinforcing actions of opiates in place preference conditioning (Bozarth and Wise 1981; Phillips et al. 1982; Spyraiki et al. 1983; Shippenberg and Herz 1987; Hand et al. 1989; however see also Mackey and van der Kooy 1985). Synergistic effects have been observed on self-stimulation behavior when morphine and amphetamine are injected together, suggesting a potent interaction between these compounds in reinforcement (Hubner et al. 1987). The opioid receptor antagonist naloxone blocks the facilitation of rate (Holtzman 1976; Franklin and Robertson 1982; Trujillo et al. 1983) and the decrease in threshold (Esposito et al. 1980) produced by amphetamine in self-stimulation and potentiates the threshold-increasing effects of chlorpromazine (Esposito et al. 1981). More recently, opiate antagonists have been found to block the cocaine-induced decrease in self-stimulation threshold (Bain and Kornetsky 1986) and to alter the self-administration of cocaine in a manner consistent with a decrease in reinforcement (Carroll et al. 1986; De Vry et al. 1989). It thus appears that opioids and catecholamines interact in positive reinforcement, and it may well be that there is an interdependence of these neurotransmitter systems in reward function (Belluzzi and Stein 1977; Maroli et al. 1978; Broekkamp et al. 1979; Bozarth and Wise 1981; Esposito et al. 1981; Bozarth 1983; Bain and Kornetsky 1986; Watson et al. 1989).

The place conditioning paradigm has attracted considerable attention in recent years as a valuable method

* A preliminary report of this research was presented at the 11th Annual Society for Neuroscience Meeting in Dallas, Texas (Trujillo et al. 1985).

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for assessing the reinforcing actions of drugs (see Bozarth 1987; van der Kooy 1987; Carr et al. 1989; Hoffman 1989 for reviews). In this paradigm, administration of a drug is paired with a distinct set of environmental cues during conditioning trials. The reinforcing or aversive properties of the drug are determined by assessing whether the subject approaches or avoids the drug-paired environment after conditioning. The place conditioning paradigm has been useful in examining the reinforcing properties of opiate drugs (Rossi and Reid 1976; Bozarth and Wise 1981; van der Kooy et al. 1982; Shippenberg and Herz 1987; Shippenberg et al. 1988, 1989), opioid peptides (Katz and Gormenzano 1979; Stapleton et al. 1979; Phillips and LePiane 1982; Glimcher et al. 1984a; Almaric et al. 1987), and psychomotor stimulants (Reicher and Holman 1977; Sherman et al. 1980; Spyraiki et al. 1982a, b; Gilbert and Cooper 1983), as well as a variety of other compounds (Glimcher et al. 1984a, b; Fudala et al. 1985; Spyraiki et al. 1985; File 1986). In addition, this method has proven valuable in studying interactions between drugs and the neurotransmitter systems they affect (Bozarth and Wise 1981; Spyraiki et al. 1982a, b; 1983; 1987; 1988; Carboni et al. 1989; Houdi et al. 1989).

In the present studies, amphetamine and naloxone were examined alone and in combination in place conditioning, in order to determine possible interactions between endogenous opioids and catecholamines in reinforcement.

Materials and methods

Animals. One hundred and forty-one experimentally naive, male, Sprague-Dawley rats (Charles River) were used. Animals weighed 250–350 g at the start of experiments, and were housed in groups of three to five in stainless steel cages on a 12 h light dark cycle, with food and water available ad lib.

Apparatus. Two identical Plexiglas shuttle boxes (80 × 25 × 30 cm), divided into three distinct compartments, were used for experiments. The shuttle boxes had clear ceilings and consisted of two large compartments (35 × 25 cm) separated by stainless steel guillotine doors from a smaller central compartment (10 × 25 cm). One of the large compartments had black walls, a stainless steel grid floor, and sawdust litter below the floor; the other had white walls, a wire mesh floor, and corncob litter below the floor. The central compartment had one black wall containing a 9 cm wide opening into the black compartment, one white wall containing a 9 cm wide opening into the white compartment, and two gray walls; guillotine doors blocking the openings could be removed to allow the animal access to the entire shuttle box. A microswitch mounted beneath the floor of each compartment detected when the animal was in that compartment. The number of entries into, and the amount of time spent within each compartment was automatically recorded by a computer interfaced with the shuttle boxes via a BRS-LVE Interact system. During experiments the testing room was dimly lit by fluorescent fixtures mounted on the ceiling. A single speaker positioned at the rear of the middle chamber delivered white noise.

Drugs. Drugs tested were *D*-amphetamine sulfate alone (1.0 mg/kg), naloxone HCl alone (0.02, 0.2, and 2.0 mg/kg), or combinations of amphetamine plus each of the three doses of naloxone, delivered in a single injection. Drugs were dissolved in sterile saline and

administered subcutaneously (SC) in a volume of 1.0 ml/kg immediately before placing the animal in the shuttle box.

General procedure. Animals were weighed and handled for at least 1 week prior to experiments. Experiments began with 3 or 4 preconditioning test days: each animal was placed in the central compartment and the guillotine doors immediately removed, giving the animal access to the entire shuttle box for 15 min. The amount of time spent by each rat in the two large compartments on the final preconditioning day was used as a measure of initial preference. The following 8 days served as the conditioning phase: on alternate days each animal was injected with drug and confined to one of the large compartments, or injected with saline and confined to the opposite compartment, for 30 min. The order of injection was counterbalanced across rats. Control animals received saline injections in both compartments. The final phase of the experiment was the postconditioning preference determination, and was identical to the preconditioning test days: each animal was placed in the central compartment (without injection) and again given access to the entire apparatus for 15 min, during which the time spent in each compartment was automatically recorded. Throughout all phases of experiments, the black compartment was wiped thoroughly with a dilute ethanol solution, and the white compartment with a dilute soap solution immediately prior to exposing each animal to the shuttle box, in order to further distinguish these compartments: the central compartment was wiped clean with distilled water in order to remove the odor of the previous animal. The conditions of the shuttle boxes established a balanced choice situation for the rats. While each rat had an individual preconditioned bias for one compartment over the other, there was no bias for the group as a whole: half the rats preferred the white compartment and half preferred the black compartment at the beginning of experiments (see Results).

Experiment 1 procedure. Amphetamine place conditioning. Amphetamine place conditioning was examined in two studies. Experiment 1a determined the ability of amphetamine to produce a preference for the initially non-preferred compartment, and compared these affects to those of saline. After the preconditioning preference determination, amphetamine-conditioned animals ($n=9$) received, on alternate days, amphetamine in the initially non-preferred compartment or saline in the initially preferred compartment. Control animals ($n=7$) received saline treatment in both compartments (the initially non-preferred compartment was designated as the drug-paired compartment for comparison with amphetamine-treated animals). Preference was determined on day 1 and on day 7 after conditioning. Experiment 1b compared amphetamine conditioning in the initially non-preferred compartment with amphetamine conditioning in the initially preferred compartment. This comparison allows one to rule out certain non-specific factors, such as a non-contingent shift in preference, that might potentially be involved in place conditioning (Spyraiki et al. 1985; Carr et al. 1989). After the preconditioning preference determination, one group of animals ($n=10$) received amphetamine in the initially non-preferred compartment and saline in the initially preferred compartment, while a second group ($n=16$) received amphetamine in the initially preferred compartment and saline in the initially non-preferred compartment, on alternate days. A third group ($n=8$) received saline in both compartments (as above, the initially non-preferred compartment was designated as the drug-paired compartment for comparison with amphetamine-treated animals).

Experiment 2 procedure. Naloxone place conditioning. This experiment examined the ability of naloxone to produce a conditioned place aversion. Following the preconditioning preference determination, animals ($n=8$ per group) received, on alternate days, naloxone (0.02, 0.2, or 2.0 mg/kg) in the initially preferred compartment or saline in the initially non-preferred compartment. For comparison and control, a fourth group received naloxone (2.0 mg/kg) in the initially non-preferred compartment, and saline in the initial-

ly preferred compartment. As noted above, this control group allows one to determine whether certain non-specific factors might play a role in the place conditioning experiment.

Experiment 3 procedure. Place conditioning with naloxone and amphetamine. Interactions between amphetamine and naloxone in place conditioning were examined in this experiment. In particular, we were interested in whether the opiate antagonist naloxone might interfere with the conditioned place preference produced by amphetamine. During conditioning, animals received amphetamine (1.0 mg/kg) and naloxone (2.0 mg/kg, $n=11$; 0.2 mg/kg, $n=8$; or 0.02 mg/kg, $n=16$) administered together in a single injection in the initially non-preferred compartment, or saline administered in the initially preferred compartment, on alternate days. For comparison and control, a fourth group ($n=24$) was conditioned with amphetamine (1.0 mg/kg) and naloxone (0.02 mg/kg) in the initially preferred compartment, alternated with saline in the initially non-preferred compartment.

Data analysis. The difference between the amount of time spent in the drug-paired compartment and the saline-paired compartment was used as the preference measure (thus, for animals conditioned in the initially non-preferred compartment, the initial preference is seen as a negative number; for animals conditioned in the initially preferred compartment the initial preference is seen as a positive number). This method of preference determination, which has been used in a number of studies (Mucha et al. 1982, 1985; Mucha and Iversen 1984; Mucha and Herz 1985; Bechara et al. 1987; Shippenberg and Herz 1987; Shippenberg et al. 1988, 1989; Bechara and van der Kooy 1989), offers an excellent graphical and statistical representation of preference and aversion in the shuttle box. Group means were obtained, and overall significance determined by two-factor repeated measures analysis of variance (drug treatment versus test day) where applicable. For individual treatments the preconditioned preference (or initial preference) was compared to the post-conditioned preference by a paired t -test. Differences between saline and drug treatments, or between different drug treatments, were compared using unpaired t -tests, or one-way analysis of variance followed by Dunnett's t -test. Reinforcing or aversive properties were determined by the ability of a drug to reverse or strengthen the initial preference of the animals for the drug-paired compartment. In addition to preference determinations, the number of entries into each compartment was quantified as a measure of locomotor activity within the apparatus.

Results

In the present studies an "unbiased" or "balanced" shuttle box was used. Although each rat individually had an initial bias, there was no overwhelming preference for one compartment over the other. This is reflected by the fact that approximately half the rats used in these experiments preferred the black compartment (77/141=55%), and approximately half preferred the white compartment (64/141=45%) prior to conditioning.

Two factor repeated measures analysis of variance of experiment 1a revealed a significant effect of drug treatment ($P<0.01$), a significant effect of test day ($P<0.001$), and a non-significant interaction ($P=0.06$). Amphetamine, paired with the initially non-preferred compartment, caused a significant shift in preference to this compartment (preconditioning = -182.1 ± 45.5 , postconditioning = 203.3 ± 72.2 , $n=9$, $P<0.001$). This preference was maintained when animals were retested after 7 unhandled days in their home cages (7 days = $222.3 \pm$

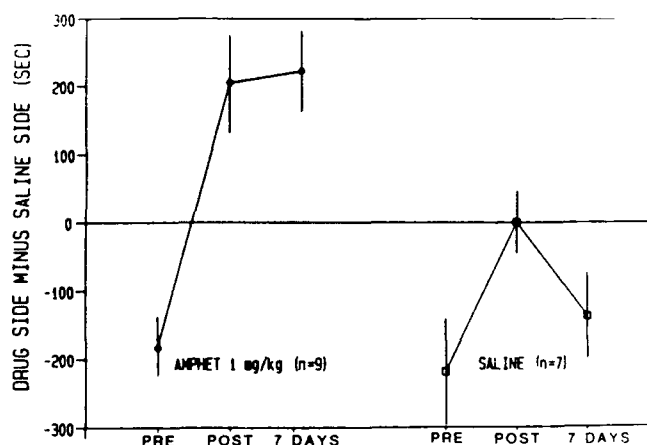


Fig. 1. Effects of amphetamine and saline in place conditioning. Amphetamine (AMPHET) paired with the initially non-preferred compartment caused animals to shift their preference to this compartment. Animals retained this altered preference when retested 7 days later. Saline paired with both compartments caused a non-significant shift to a non-preference for either compartment, which was not retained when animals were retested 7 days later. Scores represent number of seconds in the drug-paired compartment minus number of seconds in the saline-paired compartment (for saline animals, the initially non-preferred compartment was designated as the drug-paired compartment). PRE = preconditioning preference; POST = postconditioning preference.

59.1; Fig. 1). Saline, paired with both compartments caused a non-significant shift to a non-preference for either compartment; i.e., a preference of zero (preconditioning = -217.9 ± 77.4 , postconditioning = 0.6 ± 46.4 , $n=7$, n.s.). When retested after 7 days, there was a tendency for saline animals to return to preconditioned preferences, although the effect was not significant (7 days = -138.0 ± 64.6 ; Fig. 1). Unpaired t -test analyses of the saline and amphetamine group showed no significance difference between the groups at the preconditioning test, but a significant difference at the first postconditioning test ($P<0.025$), and at the 7-day test ($P<0.005$). These experiments were highly replicable - effects in experiment 1b were nearly identical to those in experiment 1a [two-factor repeated measures ANOVA: drug treatment ($P<0.001$), test day ($P<0.002$), interaction ($P<0.002$); paired t -test analysis of drug treatments: amphetamine preconditioning = -135.6 ± 27.5 , postconditioning = 189.4 ± 55.3 , $n=10$, $P<0.001$; saline preconditioning = -115.2 ± 6.3 , postconditioning = -20.6 ± 51.8 , $n=8$, n.s.; Fig. 2). When amphetamine was paired with the initially preferred compartment, no shift in preference was observed; animals maintained their preference for this compartment (preconditioning = 167.9 ± 36.9 , postconditioning = 168.1 ± 41.9 , $n=16$, n.s.; Fig. 2), demonstrating that they preferred the compartment associated with amphetamine whether it was the initially non-preferred compartment or the initially preferred compartment. Comparison of the three drug treatments on the postconditioning day (one-way ANOVA, followed by Dunnett's t -test) revealed that the saline group was significantly different from amphetamine, whether amphetamine was paired with the initially non-

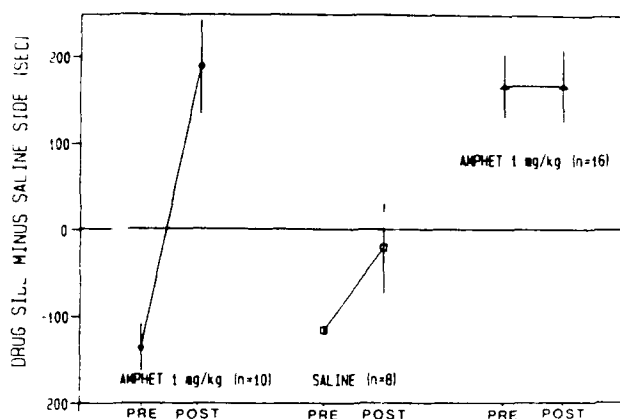


Fig. 2. Replicability of amphetamine and saline in place conditioning: effects of amphetamine conditioned in the initially preferred compartment. The effects of amphetamine (AMPHET) paired with the non-preferred compartment, and saline paired with both compartments were qualitatively and quantitatively very similar to those seen in Fig. 1 - amphetamine caused a significant shift to the drug-paired compartment, while saline caused a non-significant shift to a non-preference for either compartment. When amphetamine was paired with the initially preferred compartment, animals maintained their preference for this compartment. Scores represent number of seconds in the drug-paired compartment minus number of seconds in the saline-paired compartment (for saline animals, the initially non-preferred compartment was designated as the drug-paired compartment). *PRE* = preconditioning preference; *POST* = postconditioning preference

preferred compartment ($P < 0.025$) or paired with the initially preferred compartment ($P < 0.025$).

Naloxone caused a shift in preference away from the initially preferred compartment (Fig. 3). Two factor repeated measures analysis of variance showed no significant effect of treatment, a highly significant effect of test day ($P < 0.001$), and a significant interaction ($P < 0.005$). While 0.02 mg/kg naloxone, paired with the initially preferred compartment, did not cause a significant shift in preference (preconditioning = 98.9 ± 23.0 , postconditioning = 10.4 ± 46.3 , $n = 8$, n.s.), 0.2 mg/kg and 2.0 mg/kg produced successively greater shifts in preference away from this compartment (0.2 mg/kg preconditioning = 222.8 ± 80.4 , postconditioning = -135.0 ± 103.7 , $n = 8$, $P < 0.05$; 2.0 mg/kg preconditioning = 237.0 ± 84.8 , postconditioning = -325.2 ± 74.2 , $n = 8$, $P < 0.001$), although the difference between 0.2 and 2.0 mg/kg was not statistically significant. Naloxone (2.0 mg/kg) paired with the initially non-preferred compartment caused this compartment to be even less preferred, demonstrating that this drug produces aversion independent of the side of conditioning (preconditioning = -235.8 ± 92.6 , postconditioning = -357.2 ± 89.4 , $n = 8$, $P < 0.02$).

Animals conditioned with the combination of amphetamine (1.0 mg/kg) plus naloxone (0.02, 0.2 or 2.0 mg/kg) in the initially non-preferred compartment showed no significant change in preference (amphetamine 1.0 mg/kg plus naloxone 2.0 preconditioning = -156.3 ± 54.2 , postconditioning = -103.6 ± 72.2 , $n = 11$, n.s.; amphetamine 1.0 plus naloxone 0.2 preconditioning = -219.6 ± 50.0 , postconditioning = -157.1 ± 57.0 , $n = 8$, n.s.; amphetamine 1.0 plus naloxone 0.02 preconditioning = -133.1 ± 35.8 , postconditioning = 92.5 ± 82.2 , $n = 16$, n.s.), suggesting that naloxone interferes with the ability of amphetamine to produce a place

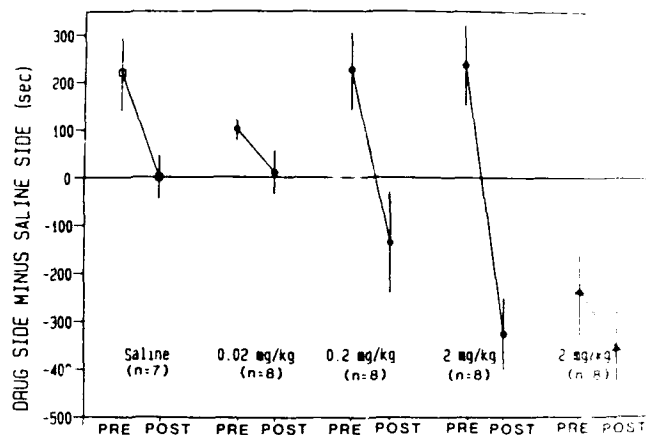


Fig. 3. Naloxone causes a dose-dependent place aversion. Saline data is the same as seen in Fig. 1, inverted for comparison with the naloxone scores (when saline is injected in both compartments either compartment may be designated as the "drug side"). The saline data is shown for visual comparison only - these data were not included in the statistical analysis. Naloxone 0.02 mg/kg paired with the initially preferred compartment did not cause a significant shift in preference. Naloxone 0.2 mg/kg and naloxone 2.0 mg/kg paired with the initially preferred compartment each caused a significant shift in preference away from this compartment. Naloxone 2.0 mg/kg paired with the initially non-preferred compartment caused this compartment to be even less preferred. Scores represent number of seconds in the drug-paired compartment minus number of seconds in the saline-paired compartment. *PRE* = preconditioning preference; *POST* = postconditioning preference

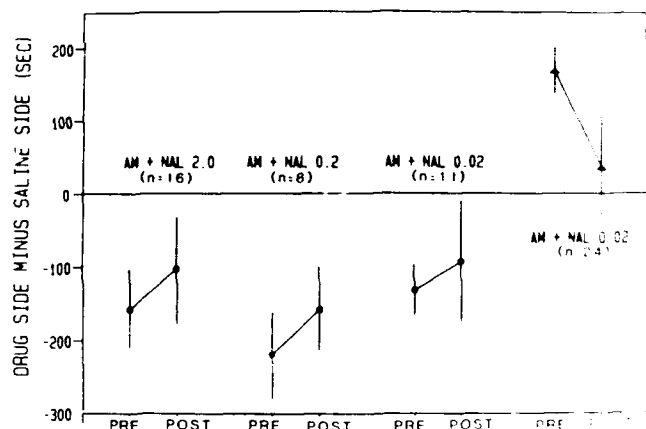


Fig. 4. Naloxone prevents the ability of amphetamine to cause a shift in place preference. When amphetamine (AM) and naloxone (NAL), administered together in a single injection, were paired with the initially non-preferred compartment, a non-significant shift toward a non-preference for either compartment was observed. When amphetamine + 1.0 mg/kg naloxone were paired with the initially preferred compartment, a non-significant shift toward a non-preference for either compartment was observed. Scores represent number of seconds in the drug-paired compartment minus number of seconds in the saline-paired compartment. *PRE* = preconditioning preference; *POST* = postconditioning preference

tioning = -219.6 ± 50.0 , postconditioning = -157.1 ± 57.0 , $n = 8$, n.s.; amphetamine 1.0 plus naloxone 0.02 preconditioning = -133.1 ± 35.8 , postconditioning = 92.5 ± 82.2 , $n = 16$, n.s.), suggesting that naloxone interferes with the ability of amphetamine to produce a place

Table 1. Effects of place conditioning on locomotor behavior. Values represent the mean number of entries \pm SEM into the drug-paired and saline-paired compartments, before and after conditioning, for each experimental treatment. The compartment which was paired with drug is shown in column 2: NPref=drug was paired with the initially non-preferred compartment; Pref=drug was paired with the initially preferred compartment (in the saline control experiment, saline was paired with both compartments, however the initially non-preferred compartment is designated as the

drug-paired compartment). The number of animals is shown in parentheses. Numbers in brackets represent the postconditioning locomotor behavior expressed as percent of preconditioning. * Significant difference ($P < 0.05$, paired *t*-test) in locomotor behavior between the preconditioning test (pre) and the postconditioning test (post). + Significant difference ($P < 0.05$, paired *t*-test) between the drug-paired and saline-paired compartments at postconditioning test

| Treatment | Conditioned compartment | | Drug | Saline |
|-------------------|-------------------------|------|------------------------|------------------------|
| Saline | Both | Pre | 14.1 \pm 1.4 | 14.8 \pm 1.3 |
| | (n = 16) | Post | 17.3 \pm 2.6 [122] | 17.7 \pm 2.3 [120] |
| Amphetamine 1.0 | NPref | Pre | 13.5 \pm 1.1 | 15.7 \pm 0.8 |
| | (n = 19) | Post | 17.2 \pm 1.2 [127] * | 15.0 \pm 1.1 [96] |
| | Pref | Pre | 15.7 \pm 1.7 | 11.6 \pm 1.6 |
| | (n = 16) | Post | 22.5 \pm 2.4 [143] * | 19.6 \pm 3.7 [169] * |
| Naloxone 0.02 | Pref | Pre | 11.2 \pm 1.4 | 11.9 \pm 1.3 |
| | (n = 8) | Post | 16.6 \pm 2.7 [148] | 18.6 \pm 3.6 [156] |
| Naloxone 0.2 | Pref | Pre | 10.6 \pm 2.0 | 10.0 \pm 2.1 |
| | (n = 8) | Post | 10.0 \pm 2.2 [94] | 15.5 \pm 3.8 [155] |
| Naloxone 2.0 | Pref | Pre | 14.1 \pm 2.0 | 11.6 \pm 2.1 |
| | (n = 8) | Post | 4.8 \pm 1.2 [34] * | 14.6 \pm 3.6 [126] † |
| | NPref | Pre | 9.2 \pm 1.5 | 11.6 \pm 2.1 |
| | (n = 8) | Post | 6.0 \pm 1.4 [65] * | 12.1 \pm 1.8 [104] † |
| Am 1.0 + Nal 0.02 | Pref | Pre | 13.8 \pm 1.2 | 10.5 \pm 1.0 |
| | (n = 24) | Post | 14.6 \pm 1.5 [106] | 13.0 \pm 1.8 [124] |
| | NPref | Pre | 14.5 \pm 2.2 | 15.1 \pm 1.7 |
| | (n = 16) | Post | 13.2 \pm 2.4 [91] | 15.6 \pm 1.8 [103] |
| Am 1.0 + Nal 0.2 | NPref | Pre | 9.9 \pm 1.9 | 14.9 \pm 3.0 |
| | (n = 8) | Post | 11.2 \pm 1.1 [113] | 19.2 \pm 2.7 [129] † |
| Am 1.0 + Nal 2.0 | NPref | Pre | 13.5 \pm 2.6 | 16.5 \pm 3.1 |
| | (n = 11) | Post | 8.3 \pm 1.8 [61] | 14.3 \pm 1.9 [87] † |

preference (Fig. 4). Note that even the lowest dose of naloxone (0.02 mg/kg), which lacked aversive effects on its own, still had the ability to block the place conditioning effects of amphetamine. When the combination of 0.02 mg/kg naloxone plus 1.0 mg/kg amphetamine was injected in the initially preferred compartment, the results were very similar to saline conditioning; the shift was toward a non-preference for either compartment (preconditioning = 168.9 ± 31.5 , postconditioning = 37.1 ± 74.3 , $n = 24$, n.s.; Fig. 4). There was no significant difference between any of the treatments at the postconditioning test.

In the present studies we assessed locomotor activity by measuring compartment entries during testing, both before and after conditioning. The number of compartment entries is not only a good measure of locomotor activity within the shuttle box, but also an excellent measure of activity within each compartment. This was demonstrated in a recent study by Neisewander et al. (1990), who found a very high correlation between the number of entries into a compartment and the number of line crossings within that compartment ($r = 0.90$, $P < 0.005$ for data shown in Table 1 of their paper). The effect of place conditioning on compartment entries for the present experiments are shown in Table 1. The general

tendency observed was a non-significant increase in total entries for most treatments, including saline control animals. These increases were typically observed in both the drug-paired and saline-paired compartments, suggesting that conditioning may lead to a mild, non-selective increase in locomotor activity within the shuttle box. Significant increases in entries into the drug-paired compartment were observed when amphetamine was paired with the initially non-preferred compartment, into both compartments when amphetamine was paired with the initially preferred compartment, and into the saline-paired compartment when amphetamine and naloxone (0.2 mg/kg) were paired with the initially non-preferred compartment. By far, the most robust effect on compartment entries was in naloxone-treated animals. The highest dose of naloxone (2.0 mg/kg) produced significant decreases in compartment entries when paired with either the initially preferred or the initially non-preferred compartment. The only treatments which produced significant differences in compartment entries between the drug-paired and saline paired compartments were naloxone (2.0 mg/kg) paired with either compartment, and naloxone (0.2 or 2.0 mg/kg) and amphetamine paired with the initially non-preferred compared. In each of these cases the drug-paired compartment had significant

cantly fewer entries than the saline-paired compartment. Thus, beyond the decrease in entries into the drug-paired compartment for animals receiving high doses of naloxone, these results demonstrate no consistent relationship between locomotor activity and place conditioning.

Discussion

Repeated pairings of a distinctive environment with amphetamine caused animals to prefer that environment over an alternative environment associated with saline, confirming previous reports of the effects of amphetamine in place conditioning (see Carr et al. 1989; Hoffman 1989 for review). The place conditioning produced by amphetamine was both highly replicable and persistent, remaining at least 7 days after conditioning. Moreover, when amphetamine was paired with the initially preferred compartment, this compartment was still preferred after conditioning. These results demonstrate that amphetamine did not cause a non-specific shift in preference, but instead that animals preferred the compartment associated with this drug regardless of whether the compartment was the initially preferred or the initially non-preferred environment. Although amphetamine did not produce an increase in preference for the initially preferred compartment, evidence suggests that the results represent a valid conditioned place preference: 1) in contrast to saline control groups a strong preference was maintained for the drug-paired compartment after conditioning, 2) the magnitude of the post-conditioning preference score was virtually identical to the score for animals conditioned with amphetamine in the initially non-preferred compartment, and 3) the results for these animals were significantly different from saline. Thus, although no increase in preference was observed for animals conditioned with amphetamine in the initially preferred compartment, the fact that the preference score remained highly positive is significant.

Interestingly, when saline was paired with both compartments, a slight, non-significant shift in preference was observed. However, this shift was not a change in preference to the opposite compartment as seen with amphetamine, but a shift to a non-preference for either compartment; i.e. a preference of zero. Although the shift was not significant in either experiment, evidence suggests that the effect is reliable. First, when the data for the two saline experiments is combined, the effect closely approaches statistical significance ($P=0.06$). Second, a similar non-significant shift was observed in animals treated with the low dose of naloxone (0.02 mg/kg) when this dose was administered alone, or when it was administered with amphetamine. The elimination of unconditioned biases with saline or very low doses of naloxone may represent habituation of the animals to the two compartments. Each animal, in the course of the experiments, was confined to each compartment for four 30-min sessions. This confinement may have led to habituation of those cues that caused the animal to prefer one environment over the other prior to injections. It is interesting to note that there was a tendency for saline-treated animals to return to preconditioned preferences

when retested 7 days later. It may be that a week without exposure to the apparatus allows the extinction of habituation and the reestablishment of unconditioned preferences. Future studies should help to elucidate the reliability and significance of the effects seen in animals receiving saline in both compartments.

Naloxone, in the present studies, caused animals to avoid the compartment associated with this drug, in a dose-dependent manner. While the effects of 0.02 mg/kg were similar to those of saline, the higher doses produced significant place aversions. In parallel with the amphetamine experiments, conditioning occurred independent of which compartment was paired with drug – animals avoided the naloxone-paired compartment whether this drug was paired with the initially preferred environment or the initially non-preferred environment, suggesting that this effect was a specific place aversion, rather than a non-specific change in compartment preference. In previous studies, conflicting results have been reported, with some studies observing place aversion with naloxone (Mucha et al. 1982, 1985; Mucha and Iversen 1984; Bechara and van der Kooy 1985; Mucha and Herz 1985), and other studies obtaining no effects of this drug in place conditioning (Phillips and LePiane 1980, 1982; Bozarth and Wise 1981). It has been suggested that the lack of effects in the latter studies resulted from insensitive procedures used by the investigators (Mucha and Iversen 1984). Significantly, the effects observed for naloxone in the present experiments were strikingly similar to those reported in two previous studies (Mucha et al. 1982; Mucha and Iversen 1984).

Animals injected with combinations of amphetamine plus naloxone in the initially non-preferred compartment showed no significant change in place preference, in an apparent blockade of amphetamine place conditioning by naloxone. However, since the 0.2 and 2.0 mg/kg doses of naloxone alone produced place aversions, it cannot be concluded that these doses simply blocked amphetamine conditioning – the interaction may have resulted from an algebraic summation of the negative effects of naloxone and the positive effects of amphetamine in place conditioning. On the other hand, since no aversive effects were detected with 0.02 mg/kg naloxone, it appears that this dose selectively blocked the place conditioning actions of amphetamine. An alternate possibility is that the combination of naloxone plus amphetamine was aversive to the animals. Despite the lack of effect of 0.02 mg/kg naloxone alone in place conditioning, it is possible that this dose in combination with amphetamine was aversive. However, animals conditioned with this combination showed effects very similar to saline – a shift toward a non-preference for either compartment, regardless of whether the conditioning took place in the initially preferred or the initially non-preferred compartment. The fact that these effects were very similar to those of saline suggests that the low dose of naloxone produced a simple blockade of amphetamine-dependent place conditioning. It is important to emphasize the low dose required for this blockade. The 0.02 mg/kg dose of naloxone is 10 fold less than the dose required to suppress self-stimulation behavior (Trullio et al.

1983, 1989a, b), and 500 fold less than the dose required to suppress locomotion (DeRossett and Holtzman 1982).

As noted above, results in place conditioning experiments are commonly interpreted as reflecting the rewarding or aversive properties of the drug(s) under study. It has been suggested, however, that the place conditioning paradigm may be confounded for drugs, such as amphetamine, which alter locomotor behavior (Swerdlow and Koob 1984). According to this suggestion, the amphetamine place preference observed in the present study may have been an artifact of increased locomotion in the drug-paired compartment. Moreover, the blockade of amphetamine place preference by naloxone may have resulted from naloxone blockade of amphetamine-dependent locomotion (Hitzemann et al. 1982; Holtzman 1974; Swerdlow et al. 1985). Several studies, however, have demonstrated that locomotor activity does not contribute significantly to place preference conditioning, and thereby dispute the suggestion that drug-induced place preferences are artifacts of alterations in locomotion (DiScala et al. 1985; Martin-Iversen et al. 1985; Mithani et al. 1986; Bozarth 1987; Vezina and Stewart 1987; Carr et al. 1988, 1989; Costello et al. 1989; Shippenberg et al. 1989). In the present studies we measured locomotion in the shuttle box during testing and found no consistent relationship between this behavior and amphetamine-induced changes in place preference. Although the present data cannot completely rule out the possibility that the place conditioning resulted from drug-induced changes in locomotion, the above noted studies, together with our data on locomotor behavior, support our suggestion that the present results are indeed a valid reflection of the motivational properties of amphetamine and naloxone, rather than a locomotor artifact. Further, although it is presently unclear whether the place conditioning paradigm measures the same aspects of reward as the self-administration or self-stimulation experiments, most investigators agree that this methodology is a legitimate tool for examining the rewarding properties of drugs (Bozarth 1987; van der Kooy 1987; Carr et al. 1989; Hoffman 1989).

Regarding possible explanations for the blockade of amphetamine reward by naloxone, it must first be considered that this effect might result from a non-specific chemical or pharmacokinetic interaction; i.e., naloxone might alter the absorption or distribution of amphetamine in the body, preventing this drug from reaching the brain. If such a mechanism were responsible for the effects of naloxone, then one might predict that this drug should similarly affect different psychoactive actions of amphetamine. However, naloxone has been reported to affect some of amphetamine's actions but not others. Holtzman (1974) observed that naloxone reduced the stimulatory effects of amphetamine on avoidance responding and locomotor activity, but not amphetamine's effects on food intake or body temperature. Likewise, Haber and coworkers (Haber et al. 1978), and Hitzemann et al. (1982) observed that naloxone selectively blocked amphetamine-stimulated rearing behavior without affecting amphetamine-dependent hyperactivity

or stereotypy. In addition, naloxone has been observed to attenuate amphetamine-dependent facilitation of dorsal tegmental self-stimulation, but not self-stimulation of the prefrontal cortex (Franklin and Robertson 1982). It should be noted that different actions of naloxone on different amphetamine-dependent behaviors does not unequivocally rule out a non-specific pharmacokinetic interaction. For example, if naloxone simply decreased the concentration of amphetamine reaching the brain, then this drug might interfere with behaviors dependent on a high dose of amphetamine, but not behaviors requiring a low dose. Nevertheless, the fact that naloxone interferes with very closely related behavioral actions of amphetamine, i.e. amphetamine-dependent rearing, but not hyperactivity or stereotypy, and selectively attenuates the effects of amphetamine on self-stimulation of one brain site but not another, lead us to believe that the present results were not due to a non-specific pharmacokinetic interaction. Moreover, if naloxone non-specifically interfered with the absorption or distribution of amphetamine in the body, then one might expect that this drug would also affect the pharmacokinetics of a variety of other drugs. However, the effects of naloxone are limited to remarkably few actions and interactions (cf. Andrews and Holtzman 1988). Naloxone blockade of amphetamine place conditioning, therefore, more likely results from a specific neural interaction between these drugs.

Although the site of interaction between naloxone and amphetamine is presently unknown, evidence suggests that the nucleus accumbens is a likely candidate. Studies suggest that amphetamine has its reinforcing action by releasing dopamine from mesolimbic nerve terminals in this nucleus (Lyness et al. 1979; Monaco et al. 1981; Spyraiki et al. 1982b; Aulisi and Hoebel 1983). Additionally, receptor binding studies have demonstrated that opioid receptors are located on mesolimbic dopamine neurons (Pollard et al. 1977). Naloxone has been observed to antagonize the amphetamine-stimulated release of ^3H -dopamine (Hitzemann et al. 1982), and the amphetamine-dependent decrease of the dopamine metabolite, homovanillic acid, in the nucleus accumbens (Applegate et al. 1982). Therefore, naloxone may prevent amphetamine reward by blocking opiate receptors on mesolimbic dopamine neurons, interfering with amphetamine-stimulated release of dopamine. Regardless of the specific neural mechanism responsible, however, the present results demonstrate that activation of opioid receptors may play an important role in the ability of amphetamine to establish a conditioned place preference.

It is notable that opiate antagonists have been observed to interfere with amphetamine in a variety of behavioral tests, including continuous avoidance responding and locomotor activity (Holtzman 1974; Swerdlow et al. 1985; Andrews and Holtzman 1987; Winslow and Miczek 1988), rearing behavior (Haber et al. 1978; Hitzemann et al. 1982), turning behavior (Dettmar et al. 1978), and acquisition and consolidation of memory (Fulginiti and Cancela 1983). More important to the present results, however, are findings of interactions be-

tween naloxone and amphetamine in self-stimulation experiments. Several investigators have reported that naloxone prevents the facilitating effect of amphetamine on self-stimulation, suggesting that blockade of opioid receptors interferes with the reinforcing actions of amphetamine (Holtzman 1976; Esposito et al. 1980; Leith 1982; Trujillo et al. 1983). The present results support this possibility, providing further evidence that activation of opioid receptors may be necessary for amphetamine reinforcement. Interestingly, recent reports examining interactions between opiate antagonists and cocaine in self-administration (Carroll et al. 1986; De Vry et al. 1989), self-stimulation (Bain and Kornetsky 1986), and place conditioning (Houdi et al. 1989) suggest that blockade of opioid receptors may interfere with the reinforcing actions of cocaine. It thus appears that opioid receptors may play a general role in the rewarding actions of psychomotor stimulants. These findings provide an interesting contrast to studies which suggest that activation of dopamine systems is necessary for opioid reinforcement (Bozarth and Wise 1981; Spyraiki et al. 1983; Shippenberg and Herz 1987; Hand et al. 1989). Despite studies demonstrating interactions between opioids and catecholamines in reward, however, other studies have found evidence against such interactions (e.g. Ettenberg et al. 1982; Mackey and van der Kooy 1985). Thus, although the evidence is not unanimous, the present results together with previous studies suggest that interactions between endogenous opioid and catecholamine systems may be important in the reinforcing actions of drugs. Further, these results hint that the neurochemistry of reward may be more complex than is currently believed.

In conclusion, the present results suggest that activation of opioid receptors is necessary for amphetamine's rewarding action. Amphetamine was observed to establish a potent conditioned place preference which could be prevented by the opiate receptor antagonist naloxone. This action of naloxone was determined to be independent of aversive effects of naloxone alone, or aversive interactions between amphetamine and naloxone, and thus appears to be a specific blockade of amphetamine reward. These results support previous studies demonstrating the ability of naloxone to block amphetamine facilitation of self-stimulation behavior (Holtzman 1976; Esposito et al. 1980; Leith 1982; Trujillo et al. 1983) and add to the increasing evidence that interactions between endogenous opioid and catecholamine systems are important in reinforcement. Moreover, in light of recent clinical findings demonstrating the potential efficacy of opiate antagonists in the treatment of cocaine abuse (Kosten et al. 1989) the present results are of particular interest, suggesting that opiate antagonists may also be effective pharmacological aids in the treatment of amphetamine abuse.

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